

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
31 January 2002 (31.01.2002)

PCT

(10) International Publication Number  
**WO 02/08265 A2**

(51) International Patent Classification<sup>7</sup>: **C07K 14/195**

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(21) International Application Number: **PCT/US01/21103**

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(22) International Filing Date: 19 July 2001 (19.07.2001)

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(25) Filing Language: English

Published:

— without international search report and to be republished upon receipt of that report

(26) Publication Language: English

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(30) Priority Data:  
60/219,361 19 July 2000 (19.07.2000) US

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**WO 02/08265 A2**

(54) Title: COMPLETE NUCLEOTIDE SEQUENCE OF *STAPHYLOCOCCUS AUREUS* RIBOSOMAL PROTEIN GENE, S20 AND METHODS FOR THE IDENTIFICATION OF ANTIBACTERIAL SUBSTANCES

(57) Abstract: The invention provides an isolated *S. aureus* ribosomal polypeptide S20, and the isolated polynucleotide molecules that encode them, vectors and host cells comprising such polynucleotide molecules and also methods for the identification of agents that effect ribosomal assembly.

**Complete Nucleotide Sequence of *Staphylococcus aureus* Ribosomal Protein Gene, S20 and Methods for the Identification of Antibacterial Substances**

**CROSS REFERENCE TO RELATED APPLICATIONS**

5       The present application claims priority of Application Serial Number  
60/219361 filed 19 July 2000 which is hereby incorporated by reference.

**FIELD OF THE INVENTION**

The present invention provides an isolated *S. aureus* S20 ribosomal polypeptide, and the isolated polynucleotide molecules that encode them, as well as  
10     vectors and host cells comprising such polynucleotide molecules. The invention also provides methods for the identification of agents that effect ribosomal assembly.

**BACKGROUND**

The staphylococci, of which *Staphylococcus aureus* is the most important human pathogen, are hardy, gram-positive bacteria that colonize the skin of most  
15     humans. Staphylococcal strains that produce coagulase are designated *S. aureus*. Other clinically important coagulase-negative staphylococci are *S. epidermidis* and *S. saprophyticus*. When the skin or mucous membrane barriers are disrupted, staphylococci can cause localized and superficial infections that are commonly  
harmless and self-limiting. However, when staphylococci invade the lymphatics and  
20     the blood, potentially serious complications may result, such as bacteremia, septic shock, and serous metastatic infections, including endocarditis, arthritis, osteomyelitis, pneumonia and abscesses in virtually any organ. Certain strains of *S. aureus* produce toxins that cause skin rashes, food poisoning, or multisystem dysfunction (as in toxic shock syndrome). *S. aureus* and *S. epidermidis* together have  
25     become the most common cause of nosocomial non-urinary tract infection in U.S. hospitals. They are the most frequently isolated pathogens in both primary and secondary bacteremias and in cutaneous and surgical wound infections. See generally *Harrison's Principles of Internal Medicine*, 13<sup>th</sup> ed., Isselbacher et. al. eds. McGraw-Hill, New York (1994), particularly pages 611-617.

30       Transient colonization of the nose by *S. aureus* is seen in 70-90 percent of people, of which 20 to 30 percent carry the bacteria for relatively prolonged periods of time. Independent colonization of the perineal area occurs in 5-20 percent of people. Higher carriage rates of *S. aureus* have been documented in persons with atopic

dermatitis, hospital employees, hospitalized patients, patients whose care requires frequent puncture of the skin, and intravenous drug abusers.

Infection by staphylococci usually results from a combination of bacterial virulence factors and a diminution in host defenses. Important microbial factors 5 include the ability of the staphylococcus to survive under harsh conditions, its cell wall constituents, the production of enzymes and toxins that promote tissue invasion, its capacity to persist intracellularly in certain phagocytes, and its potential to acquire resistance to antimicrobials. Important host factors include an intact mucocutaneous barrier, and adequate number of functional neutrophils, and removal of foreign bodies 10 or dead tissue.

Once the skin or mucosa have been breached, local bacterial multiplication is accompanied by inflammation, neutrophil accumulation, tissue necrosis, thrombosis and fibrin deposition at the site of infection. Later, fibroblasts create a relatively avascular wall about the area. When host mechanisms fail to contain the cutaneous or 15 submucosal infection, staphylococci may enter the lymphatics and the bloodstream. Common sites of metastatic spread include the lungs, kidneys, cardiac valves, myocardium, liver, spleen, bone and brain.

Antimicrobial resistance by staphylococci favors their persistence in the hospital environment. Over 90 percent of both hospital and community strains of *S. 20 aureus* causing infection are resistant to penicillin. This resistance is due to the production of  $\beta$  lactamase enzymes. The genes for these enzymes are usually carried by plasmids. Infections due to organisms with such acquired resistance can sometimes be treated with  $\beta$  lactamase resistant penicillin derivatives. However the true penicillinase-resistant *S. aureus* organisms, called methicillin resistant *S. aureus* 25 (MRSA), are resistant to all the  $\beta$  lactam antibiotics and the cephalosporins. MRSA resistance is chromosomally mediated and involves production of an altered penicillin-binding protein (PBP 2a or PBP 2') with a low binding for  $\beta$  lactams. MRSA frequently also have acquired plasmids mediating resistance to erythromycin, tetracycline, chloramphenicol, clindamycin, and aminoglycosides. MRSA have 30 become increasingly common worldwide, particularly in tertiary-care referral hospitals. In the United States, approximately 32 percent of hospital isolates of *S. aureus* are methicillin resistant. Methicillin resistant staphylococci are a serious clinical and economic problem, since treatment of these infections often requires

vancomycin, an antibiotic that is more difficult to administer and more expensive than the penicillins. Quinolone antimicrobial agents have been used to treat methicillin-resistant staphylococcal infections. Unfortunately, resistance to these antibiotics has also developed rapidly. Sixty to 70% of methicillin resistant *S. aureus* isolates are  
5 also quinolone resistant.

A pressing need exists for new chemical entities that are effective in the treatment of staphylococcal infections. One fruitful area of research has been in the area of agents which inhibit protein synthesis. A large number of antibacterial agents, including many in current clinical use, inhibit protein synthesis in bacteria by  
10 interfering with essential functions of the ribosome. When ribosomal function is perturbed, protein synthesis may cease entirely or, alternatively, it may be sufficiently slowed so as to stop normal cell growth and metabolism. Differences between the prokaryotic 70S ribosomes (composed of 50S and 30S subunits) and the eukaryotic 80S ribosome (composed of 60S and 40S subunits) underlie the basis for the selective  
15 toxicity of many antimicrobial agents of this class. However, a limited subset of this class of antimicrobial agents exhibits some cross-reactivity with the 70S ribosomes of eukaryotic mitochondria. This cross-reactivity probably accounts for the host cells cytotoxicity effects observed with some agents and has limited their use as clinical antimicrobial agents. Other agents (e.g., tetracycline), which affect the function of  
20 eukaryotic 80S ribosomes in vitro, are still used clinically to treat bacterial infections as the concentrations employed during antimicrobial therapy are not sufficient to elicit host cell toxicity side-effects.

Moreover, protein biosynthesis inhibitors can be divided into a number of different classes based on differences in their mechanisms of action. The  
25 aminoglycoside agents (e.g., streptomycin) bind irreversibly to the 30S subunit of the ribosome, thereby slowing protein synthesis and causing mis-translation (i.e., mis-reading) of the mRNA. The resulting errors in the fidelity of protein synthesis are bacteriocidal, and the selective toxicity of this family of agents is increased by the fact that bacteria actively transport them into the cell.  
30 The tetracycline family of agents (e.g., doxycycline) also binds to the 30S ribosome subunit, but does so reversibly. Such agents are bacteriostatic and act by interfering with the elongation phase of protein synthesis by inhibiting the transfer of the amino acid moieties of the aminoacyl-tRNA substrates into the

growing polypeptide chain. However, inhibition mediated by the tetracyclines is readily reversible, with protein synthesis resuming once intracellular levels of the agent's decline. Chloramphenicol and the macrolide family of agents (e.g., erythromycin), in contrast, act on the function/activity of the 50S subunit of  
5 the ribosome. These agents are bacteriostatic in nature, and their effects are reversible. It has also been suggested that both chloramphenicol and the macrolides may have a second mode of action involved in ribosomal assembly. Champney and Burdine (1995). Finally, puromycin acts as a competitive inhibitor of the binding of aminoacyl-tRNA's to the so-called aminoacyl site (i.e., A-site) of the ribosome and  
10 acts as a chain-terminator of the elongation phase as a result of its incorporation into the growing peptide chain.

It has been shown in *E. coli* that mutants which lack S20 in ribosomes , as judged by 2-dimensional electrophoresis are impaired in 30S subunit association with 50S subunits to form 70S ribosomes. Ryden-Aulin et al. (1993) Molecular  
15 Microbiology 7(6) 983-992. The mutants described by Ryden-Aulin misread nonsense codons and show a greatly reduced growth rate. Because of this growth impairment S20 ribosomal polypeptide is an attractive molecular target for the development of antibacterial agents effective against *S. aureus* and related organisms. It has also been noted that mitochondrial ribosomes lack a homolog of  
20 the bacterial S20 protein. Koc et al. (2001) J. Biol. Chem 276 (22) 19363-19374. The lack of a mitochondrial counterpart makes S20 even more attractive as a bacteria-specific target.

This document discloses important new methods of identifying antibacterial substances related to the bacterial ribosomal assembly process, and to the  
25 *Staphylococcal* ribosomal protein S20 and it for the first time discloses the full nucleotide and amino acid sequence of *Staphylococcus aureus* S20 ribosomal polypeptide

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20 **Brief Description of the Sequence Listings**

SEQ ID NO:1 Complete coding sequence of S20 ribosomal polypeptide

SEQ ID NO:2 Predicted polypeptide sequence of S20 ribosomal polypeptide

SEQ ID NO:3 Sequencing Primer

SEQ ID NO:4 Sequencing Primer

25 SEQ ID NO:5 Sequencing Primer

SEQ ID NO:6 Sequencing Primer

SEQ ID NO:7 Sequencing Primer

SEQ ID NO:8 Sequencing Primer

SEQ ID NO:9 PCR Primer

30 SEQ ID NO:10 PCR Primer

SEQ ID NO:11 DNA sequence for *Staphylococcus aureus* S4 ribosomal protein gene (coding and flanking sequences)

SEQ ID NO:12 Polypeptide sequence for *Staphylococcus aureus* S4 ribosomal protein

SEQ ID NO:13 DNA sequence for *Staphylococcus aureus* S7 ribosomal protein gene

35 (coding and flanking sequences)

SEQ ID NO:14 Polypeptide sequence for *Staphylococcus aureus* S7 ribosomal protein

SEQ ID NO:15 DNA sequence for *Staphylococcus aureus* S8 ribosomal protein gene (coding and flanking sequences)

SEQ ID NO:16 Polypeptide sequence for *Staphylococcus aureus* S8 ribosomal protein

SEQ ID NO:17 DNA sequence for *Staphylococcus aureus* S15 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:18 Polypeptide sequence for *Staphylococcus aureus* S15 ribosomal protein

5 SEQ ID NO:19 DNA sequence for *Staphylococcus aureus* S17 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:20 Polypeptide sequence for *Staphylococcus aureus* S17 ribosomal protein

10 SEQ ID NO:21 DNA sequence for *Staphylococcus aureus* 16S ribosomal RNA gene  
(coding and flanking sequences)

SEQ ID NO:22 DNA sequence for *Staphylococcus aureus* S1 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:23 Polypeptide sequence for *Staphylococcus aureus* S1 ribosomal protein gene

15 SEQ ID NO:24 DNA sequence for *Staphylococcus aureus* S2 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:25 Polypeptide sequence for *Staphylococcus aureus* S2 ribosomal protein

SEQ ID NO:26 DNA sequence for *Staphylococcus aureus* S3 ribosomal protein gene  
(coding and flanking sequences)

20 SEQ ID NO:27 Polypeptide sequence for *Staphylococcus aureus* S3 ribosomal protein

SEQ ID NO:28 DNA sequence for *Staphylococcus aureus* S5 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:29 Polypeptide sequence for *Staphylococcus aureus* S5 ribosomal protein

SEQ ID NO:30 DNA sequence for *Staphylococcus aureus* S6 ribosomal protein gene

25 (coding and flanking sequences)

SEQ ID NO:31 Polypeptide sequence for *Staphylococcus aureus* S6 ribosomal protein

SEQ ID NO:32 DNA sequence for *Staphylococcus aureus* S9 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:33 Polypeptide sequence for *Staphylococcus aureus* S9 ribosomal protein

30 SEQ ID NO:34 DNA sequence for *Staphylococcus aureus* S10 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:35 Polypeptide sequence for *Staphylococcus aureus* S10 ribosomal protein

SEQ ID NO:36 DNA sequence for *Staphylococcus aureus* S11 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:37 Polypeptide sequence for *Staphylococcus aureus* S11 ribosomal protein

5 SEQ ID NO:38 DNA sequence for *Staphylococcus aureus* S12 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:39 Polypeptide sequence for *Staphylococcus aureus* S12 ribosomal protein

10 SEQ ID NO:40 DNA sequence for *Staphylococcus aureus* S13 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:41 Polypeptide sequence for *Staphylococcus aureus* S13 ribosomal protein

15 SEQ ID NO:42 DNA sequence for *Staphylococcus aureus* S14 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:43 Polypeptide sequence for *Staphylococcus aureus* S14 ribosomal protein

20 SEQ ID NO:44 DNA sequence for *Staphylococcus aureus* S16 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:45 Polypeptide sequence for *Staphylococcus aureus* S16 ribosomal protein

25 SEQ ID NO:46 DNA sequence for *Staphylococcus aureus* S18 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:47 Polypeptide sequence for *Staphylococcus aureus* S18 ribosomal protein

30 SEQ ID NO:48 DNA sequence for *Staphylococcus aureus* S19 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:49 Polypeptide sequence for *Staphylococcus aureus* S19 ribosomal protein

SEQ ID NO:50 DNA sequence for *Staphylococcus aureus* S20 ribosomal polypeptide gene  
(coding and flanking sequences)

35 SEQ ID NO:51 DNA sequence for *Staphylococcus aureus* S21 ribosomal protein gene  
(coding and flanking sequences)

SEQ ID NO:52 Polypeptide sequence for *Staphylococcus aureus* S21 ribosomal protein

SEQ ID NO:53 Exemplary S4 Forward PCR Primer

SEQ ID NO:54 Exemplary S4 Reverse PCR Primer

5 SEQ ID NO:55 Exemplary S18 Forward PCR Primer

SEQ ID NO:56 Exemplary S18 Reverse PCR Primer

SEQ ID NO:57 Exemplary S6 Forward PCR Primer

SEQ ID NO:58 Exemplary S6 Reverse PCR Primer

SEQ ID NO:59 Exemplary 16S H-44 Helical RNA Forward PCR Primer

10 SEQ ID NO:60 Exemplary 16S H-44 Helical RNA Reverse PCR Primer

SEQ ID NO:61 Exemplary 16S H-7, 8,9,10 & 11 Helical RNA Forward PCR Primer

SEQ ID NO:62 Exemplary 16S H-7, 8,9,10 & 11 Helical RNA Reverse PCR Primer

**Brief Description of the Figures**

Figure 1- DNA Coding Region and Amino Acid Sequence of the S20 ribosomal polypeptide

15 Figure 2. Column Profile of HiPrep SP<sub>XL</sub> Column

Figure 3. Coomassie-stained NuPage Gels of S20 ribosomal polypeptide fractions. Using Novex NuPage™ Bis-gels Tris (4-12%) with a MES Buffer system

Figure 4 Graphic illustration of how specific inhibition of S20 ribosomal polypeptide binding to RNA is detected.

20 Figure 5 Graphic illustration of a ribosomal assembly map incorporating direct binding S proteins (S4, S8, S7, S17, and S20) as well as some proteins which integrate themselves into ribosomes by reliance on protein-protein interactions (non-direct binding proteins) (S3, S5, S9, S10, S12, S14, S16 and S19). Arrows between proteins indicate the effect of a protein on another whose binding it enhances. Thick arrows indicate a principal contribution. Thin arrows indicate lesser contribution. Noller and Nomura (1987)

25 Figure 6 Graphical illustration of a ribosomal assembly assay incorporating direct binding S proteins (S4, S8, S7, S17, and S20) as well as proteins which integrate themselves into ribosomes by reliance on protein-protein interactions “non direct binding proteins”(S3, S5, S9, S10, S12, S14, S16 and S19).

## SUMMARY OF THE INVENTION

The present invention provides an isolated *S aureus* S20 ribosomal polypeptide, and the isolated polynucleotide molecules that encode them, as well as vectors and host cells comprising such polynucleotide molecules. The DNA sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded polypeptide, upon expression, can be used as a target for the screening of antibacterial drugs. High-throughput assays for identifying inhibitors of ribosomal assembly are provided. Solid phase high throughput assays are provided, as are related assay compositions, integrated systems for assay screening and other features that will be evident upon review.

In one embodiment, the invention provides an isolated S20 ribosomal polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2. The DNA and predicted amino acid sequence of *Staphylococcus aureus* S20 ribosomal polypeptide is displayed below:

```

ATGGCAAATATCAAATCTGCAATTAAACGTGTAAAAACAACGTGAAAAAGCTGAAGCACGC60
M A N I K S A I K R V K T T E K A E A R

20 AACATTCACAAAAGAGTGCAATGCGTACAGCAGTTAAAACGCTAAAACAGCTGTTCA120
N I S Q K S A M R T A V K N A K T A V S

AATAACGCTGATAATAAAAATGAATTAGTAAGCTTAGCAGTTAAGTTAGTAGACAAAGCT180
N N A D N K N E L V S L A V K L V D K A
25 GCTCAAAGTAATTAAATACATTCAAACAAAGCTGACCGTATTAAATCACAATTAAATGACT240
A Q S N L I H S N K A D R I K S Q L M T

GCAAATAAATAA252
30 A N K *

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Although SEQ ID NOS:1 and 2 provide particular *S. aureus* sequences, the invention is intended to include within its scope other *S. aureus* allelic variants. Allelic variants are understood to mean naturally-occurring base changes in the species population which may or may not result in an amino acid change of the DNA sequences herein

The present invention also includes variants of the aforementioned polypeptide, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics.

5       The nucleic acids of the invention include those nucleic acids coding for the same amino acids in the S20 ribosomal polypeptide due to the degeneracy of the genetic code

In another embodiment, the invention provides isolated polynucleotides (e.g. RNA and DNA, both naturally occurring and synthetically derived, both single and double stranded) that comprise a nucleotide sequence encoding the amino acid sequence of the polypeptides of the invention. Such polynucleotides are useful for recombinantly expressing the enzyme and also for detecting expression of the polypeptides in cells (e.g. using Northern hybridization and in situ hybridization assays). Specifically excluded from the definition of polynucleotides of the invention 10 is the entire isolated chromosome of the native host cells. A preferred polynucleotide of the invention set forth in SEQ ID NO:1 corresponds to the naturally occurring S20 ribosomal polypeptide encoding nucleic acid sequence. It will be appreciated that numerous other sequences exist that also encode S20 ribosomal polypeptide of SEQ 15 ID NO:2 due to the well known degeneracy of the universal genetic code. In another preferred embodiment the invention is directed to all isolated degenerate 20 polynucleotides encoding the S20 ribosomal polypeptide.

In another embodiment the invention provides an isolated nucleic acid comprising the nucleotide sequence having least 60%, 70%, 80, 90% identity with SEQ ID NO:1. In one embodiment, the invention provides an isolated S20 ribosomal 25 polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

In a related embodiment the invention provides vectors comprising a polynucleotide of the invention. Such vectors are useful, e.g. for amplifying the polynucleotides in host cells to create useful quantities thereof. In preferred embodiments, the vector is an expression vector wherein the polynucleotide of the 30 invention is operatively linked to a polynucleotide comprising an expression control sequence. Such vectors are useful for recombinant production of polypeptides of the invention.

In another related embodiment, the invention provides host cells that are transformed with polynucleotides or vectors of the invention. As stated above, such host cells are useful for amplifying the polynucleotides and also for expressing the S20 ribosomal polypeptide or a fragment thereof encoded by the polynucleotide.

5 In still another related embodiment, the invention provides a method for producing the S20 ribosomal polypeptide (or a fragment thereof) comprising the steps of growing a host cell of the invention in a nutrient medium and isolating the S20 ribosomal polypeptide from the cells.

10 In still another related embodiment, the invention provides a method for testing for inhibitors of ribosomal assembly comprising the steps of contacting a labeled S20 ribosomal polypeptide with a ribosomal RNA in the presence and the absence of a test agent, determining the amount of S20 ribosomal polypeptide specifically bound to said RNA both in the presence of a test agent and in the absence of said test agent, and comparing the amount of protein determined in the presence of 15 the test agent to the amount of protein determined in step in the absence of the test agent.

A decrease in the amount of protein determined in the presence of test agent compared to that determined in the absence of the test agent indicates that said agent is an inhibitor of ribosomal assembly

20 In still another related embodiment, the invention provides a method for testing for inhibitors of ribosomal assembly comprising the steps of contacting at least one direct binding ribosomal polypeptide selected from the group consisting of S4, S7, S8, S15, S17 and S20 with 16S ribosomal RNA in the presence and absence of a test agent and determining the amount of direct binding protein bound to the RNA in 25 the presence of a test agent; and in the absence of said test agent; and comparing the amount direct binding protein determined under both sets of conditions.

A decrease in the amount of direct binding protein determined in the presence of test agent compared to that determined in the absence of the test agent indicates that said agent is an inhibitor of ribosomal assembly

30 In still another related embodiment the invention provides a method for testing for inhibitors of ribosomal assembly comprising the steps of contacting at least one direct binding ribosomal polypeptide selected from the group consisting of S4, S7, S8, S15, S17 and S20 with 16S ribosomal RNA to form a polyribonucleotide protein

complex and; contacting said polyribonucleotide protein complex with at least one non- direct binding ribosomal polypeptide selected from the group consisting of S1, S2, S3, S5, S6, S9, S10, S11, S12, S13, S14, S16, S18, S19, and S21.

in the presence and absence of a test agent; and then determining the amount of at  
5 least one non- direct binding ribosomal polypeptide bound to the RNA  
in the presence and the absence of a test agent and then comparing the amount of  
least one non direct binding ribosomal polypeptide bound under both conditions

In still another related embodiment the invention provides an isolated S20  
ribosomal polypeptide comprising an amino acid sequence at least 70%, 80, 90%,  
10 95% identical to the sequence of SEQ ID NO:2.

In addition to the foregoing, the invention includes as an additional aspect, all  
embodiments of the invention narrower in scope in any way than the variations  
specifically mentioned above. Although the applicant(s) invented the full scope of the  
claims appended hereto, the claims appended are not intended to encompass within  
15 their scope the prior art work of others. Therefore, in the event that statutory prior art  
within the scope of a claim is brought to the attention of the applicants by a Patent  
Office or other entity or individual, the applicant(s) reserve the right to exercise  
amendment rights under applicable patent laws to redefine the subject matter of such a  
claim to specifically exclude such statutory prior art or obvious variations of statutory  
20 prior art from the scope of such a claim. Variations of the invention defined by such  
amended claims also are intended as aspects of the invention.

#### **DETAILED DESCRIPTION OF THE INVENTION**

The foregoing is provided to further facilitate understanding of the applicant's  
invention but is not intended to limit the scope of applicant's invention.

25 **Definitions**

As used hereinafter "Isolated" means altered by the hand of man from the  
natural state. If an "isolated" composition or substance occurs in nature, it has been  
changed or removed from its original environment, or both. For example, a  
polynucleotide or a polypeptide naturally present in a living animal is not "isolated,"  
30 but the same polynucleotide or polypeptide separated from the coexisting materials of  
its natural state is "isolated", as the term is employed herein.

As used hereinafter "Polynucleotide" generally refers to any  
polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or

DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that

5 may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include,

10 for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short

15 polynucleotides, often referred to as oligonucleotides.

As used hereinafter "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a

heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation,  
5 gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, Proteins-Structure and Molecular Properties, 2nd Ed., T. E.  
10 Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., Post-translational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in Postranslational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", Meth Enzymol (1990) 182:626-646 and Rattan et al., "Protein  
15 Synthesis: Post-translational Modifications and Aging", Ann NY Acad Sci (1992) 663:4842).

As used hereinafter "Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, 20 reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid 25 sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one 30 encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

As used hereinafter "Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques (see, e.g.: *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A. M., and Griffin, H. G., eds., Humana Press, N.J., 1994; *Sequence Analysis in Molecular Biology*, von Heijne, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., SIAM J Applied Math (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J Applied Math (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, and FASTA (Atschul, S. F. et al., *J Molec Biol* (1990) 215:403). The well known Smith Waterman algorithm may be used to determine identity. The Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison, Wisconsin) is one such program which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.* 2:482-489 (1981)).

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in

one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity(divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID

5 NO:1, or:

$$n_n \leq x_n - (x_n \cdot y)$$

wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of 10 nucleotides in SEQ ID NO:1, and  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or 15 frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference 20 sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed 25 either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity(divided by 100) and then subtracting that product from said total number of 30 amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y)$$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2, and  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ . Identity has been similarly defined in 5 US Patent No. 6,083,924 which is hereby incorporated by reference.

The present invention provides isolated polynucleotides (e.g., DNA sequences and RNA transcripts, both sense and complementary antisense strands, both single and double stranded) encoding a *Staphylococcus aureus* ribosomal protein S20. The nucleic acids of the invention include those nucleic acids coding for the same amino acids in the S20 ribosomal polypeptide due to the degeneracy of the genetic code. 10

DNA polynucleotides of the invention include genomic DNA and DNA that has been synthesized in whole or in part. "Synthesized" as used herein and understood in the art, refers to polynucleotides produced by purely chemical as opposed to enzymatic methods. "Wholly" synthesized DNA sequences are therefore produced entirely by 15 chemical means, and "partially" synthesized DNAs embrace those wherein only portions of the resulting DNA were produced by chemical means.

Genomic DNA of the invention comprises the protein-coding region for a polypeptide of the invention and is also intended to include allelic variants. Allelic variants. Allelic variants are understood to mean naturally-occurring base changes in 20 the species population which may or may not result in an amino acid change of the DNA sequences herein.

"16S ribosomal RNA" is understood to mean an isolated small subunit RNA of any prokaryote whether isolated from ribosomes, made synthetically or prepared by transcription, "16S ribosomal RNA" can mean either the full length sequence or a 25 fragment thereof.

As used herein, the term "contacting" means bringing together, either directly or indirectly, a compound into physical proximity to a polypeptide or polynucleotide of the invention. Additionally "contacting" may mean bringing a polypeptide of the invention into physical proximity with another polypeptide or polynucleotide (either 30 another polypeptide or polynucleotide of the invention or a polypeptide or polynucleotide not so claimed) or bringing a polynucleotide of the invention into physical proximity with a polypeptide or polynucleotide (either a polypeptide or polynucleotide of the invention or a polypeptide or polynucleotide not so claimed).

As used herein, the term "polyribonucleotide protein complex" refers to a covalent or non-covalently associated molecular entity containing 16S ribosomal RNA and at least one small subunit ribosomal protein

"Small subunit ribosomal protein" as used herein refers to ribosomal proteins present in the small (30S) ribosomal subunit of the ribosome of derived from any prokaryotic species. Small subunit ribosomal proteins include: S1, S2 S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, and S21.

"Direct binding ribosomal polypeptide" or "direct binding S-protein" or "direct binding ribosomal protein" or "direct binding protein" as used herein refers to a polypeptide derived from any prokaryotic species selected from the group consisting of S4, S7, S8, S17, S15 and S20

"Non- direct binding ribosomal polypeptide" or "non direct binding S-protein" or "non direct binding ribosomal protein" or "non-direct binding protein" as used herein refers to a polypeptide derived from any prokaryotic species selected from the group consisting of S1, S2 S3, S5, S6, S9, S10, S11, S12, S13, S14, S16, S18, S19, and S21. These proteins are also referred to as "secondary binding proteins".

"Antibodies" as used herein includes monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and humanized antibodies, as well as Fab fragments, including the products of an Fab immunoglobulin expression library. 20 The S20 ribosomal polypeptides of the invention or variants thereof, or cells expressing them can be used as an immunogen to produce antibodies immunospecific for such polypeptides.

#### Nucleic Acids of the Invention

A preferred DNA sequence of the invention encoding the *Staphylococcus aureus* S20 ribosomal polypeptide is set out in SEQ ID NO:1. The worker of skill in the art will readily appreciate that the preferred DNA of the invention comprises a double stranded molecule, for example the molecule having the sequence set forth in SEQ ID NO:1 along with the complementary molecule (the "non-coding strand" or "complement") having a sequence deducible from the sequence of SEQ ID NO:1 according to Watson-Crick base pairing rules for DNA. Also preferred are other polynucleotides encoding the S20 ribosomal polypeptide of SEQ ID NO:2, which differ in sequence from the polynucleotide of SEQ ID NO:1 by virtue of the well-

known degeneracy of the universal genetic code. The determination of the nucleotide sequence is described in the following example.

### Example 1

5   **Procedure for obtaining sequence information of the S20 gene directly from the  
2.8 Mb *S. aureus* genome.**

The *S. aureus* S20 gene was sequenced using an ABI377 fluorescence-based sequencer (Perkin Elmer/Applied Biosystems Division, PE/ABD, Foster City, CA) and the ABI PRISM™ Ready Dye-Deoxy Terminator kit with *Taq* FS™ polymerase.

10   Each ABI cycle sequencing reaction contained about 4 µg of Qiagen purified *S. aureus* genomic DNA, 100 ng of primer, and in a 2X standard reaction volume (40 µl total volume). Cycle-sequencing was performed using an initial denaturation at 98°C for 1 min, followed by 100 cycles: 98°C for 30 sec, annealing at 50°C for 30 sec, and extension at 60°C for 4 min. Temperature cycles and times were controlled by a

15   Perkin-Elmer 9700 thermocycler. Extension products were purified using Centriflex™ gel filtration cartridges (Advanced Genetic Technologies Corp., Gaithersburg, MD). Each reaction product was loaded by pipette onto the column, which was then centrifuged in a swinging bucket centrifuge (Sorvall model RT6000B table top centrifuge) at 1500 x g for 4 min at room temperature. Column-purified

20   samples were dried under vacuum for about 40 min and then dissolved in 1.5 µl of a DNA loading solution (83% deionized formamide, 8.3 mM EDTA, and 1.6 mg/ml Blue Dextran). The samples were then heated to 90°C for three min and the complete sample was loaded into the gel sample well of the ABI377 sequencer. Sequence analysis was done by importing ABI377 files into the Sequencher program (Gene

25   Codes, Ann Arbor, MI). Generally sequence reads of 600 bp were obtained. Sequence base call ambiguities were removed by obtained the complete sequence of each gene on both DNA strands.

#### Sequencing of the *S. aureus* S20 gene.

Partial DNA sequences encoding a portion of *S. aureus* S20 ribosomal polypeptide have been described. Human Genome Sciences ID #V76479 and TIGR # TI:GSA\_604. The TIGR sequence matches the first 79 nucleotides of the sequence disclosed in this invention. The Human Genome Sciences, Inc. sequence contains 109 nucleotides which codes for the carboxy terminal 35 amino acid residues. The

combination of the TIGR and HGS partial S20 ribosomal polypeptide gene sequences do not overlap as they contain a 63 nucleotide gap. The invention provides a complete sequence. The *Bacillus subtilis* ribosomal S20 polypeptide shares some identity with the *S. aureus* S20 ribosomal polypeptide; however the proteins differ by 5 about 52% identity in their protein sequences.

The 187 bp GST in the TIGR database (TI:GSA\_604) encodes about 26 amino acids of the *S. aureus* S20 ribosomal polypeptide gene. starting with the Met codon. This sequence, of unknown quality, was used to design three forward primers, SEQ ID NO:3 (5'AATATCAAATCTGCAATTAAACG)  
10 SEQ ID NO:4 (5'AAATTTGATAAGATGAACTCAC) and SEQ ID NO:5 (5'TTTAGGAGGTGACAGAAATGGC). Only one of these primers generated any useful new sequence data, SEQ ID NO:3 primed a poor sequence read of about 400 bp. A second attempt using primer SEQ ID NO:3 produced a higher quality read that extended about 600 bp. Both reads were used to design three  
15 additional primers, forward primer SEQ ID NO:6 .  
(5'ACGCAACATTCACAAAAGAGTGC)  
and reverse primer SEQ ID NO:7 (5'- ATTGCACTCTTTGTGAAATGTTGC) and SEQ ID NO:8 (5'- ATCTTATAAAAAATAAAAGTTC). Excellent sequence reads of more than 500 bp. were obtained from primers SEQ ID NO:6 and SEQ ID NO:7  
20 and a poor quality, but usable, read was obtained from primer SEQ ID NO:8. The combined four reads provided the complete double-stranded sequence of the *S.aureus* S20 ribosomal polypeptide gene region. Thus, the goal to obtain the complete accurate sequence of the *S. aureus* S20 ribosomal polypeptide gene directly from the genome was achieved. A total of 1.2 kb of sequence data was obtained within and  
25 around the S20 ribosomal polypeptide gene.

The invention further embraces species, which are homologs of the *Staphylococcus aureus* S20 ribosomal polypeptide encoding DNA. Species homologs, would encompass nucleotide sequences which share at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 30 at least 98%, at least 99% identity with *Staphylococcus aureus* polynucleotide of the invention

The polynucleotide sequence information provided by the invention makes possible large scale expression of the encoded polypeptide by techniques well known

and routinely practiced in the art. Polynucleotides of the invention also permit identification and isolation of polynucleotides encoding related ribosomal proteins, such as allelic variants and species homologs, by well known techniques including Southern and/or Northern hybridization, and polymerase chain reaction (PCR).

5       The disclosure herein of a full length polynucleotide encoding an S20 ribosomal polypeptide makes readily available to the worker of ordinary skill in the art every possible fragment of the full length polynucleotide. The invention therefore provides fragments of the S20 ribosomal polypeptide encoding polynucleotides comprising at least 14-15, and preferably at least 18, 20, 25, 50, or 75 consecutive 10 nucleotides of a polynucleotide encoding S20 ribosomal polypeptide. Preferably, fragment polynucleotides of the invention comprise sequences unique to the S20 ribosomal polypeptide encoding polynucleotide sequence and therefore hybridize under highly stringent or moderately stringent conditions only (i.e. "specifically") to polynucleotides encoding S20 ribosomal polypeptide. Sequences unique to 15 polynucleotides of the invention are recognizable through sequence comparison to other known polynucleotides, and can be identified through use of alignment programs routinely utilized in the art, e.g. those made available in public sequence databases. Such sequences are also recognizable from Southern hybridization analyses to determine the number of fragments of genomic DNA to which a 20 polynucleotide will hybridize. Polynucleotides of the invention can be labelled in a manner that permits their detection , including radioactive, fluorescent, and enzymatic labelling.

Fragment polynucleotides are particularly useful as probes for detection of full length or other fragment S20 ribosomal polypeptide polynucleotides or for the 25 expression of fragments of S20 ribosomal polypeptide. One or more fragment polynucleotides can be included in kits that are used to detect variations in a polynucleotide sequence encoding S20 ribosomal polypeptide.

The invention also embraces DNAs encoding S20 ribosomal polypeptide polypeptides which DNAs hybridize under moderately stringent or high stringency 30 conditions to the non-coding strand, or complement, of the polynucleotide in SEQ ID NO:1

Exemplary highly stringent hybridization conditions are as follows:  
hybridization at 42°C in a hybridization solution comprising 50% formamide, 1%

SDS, 1M NaCl, 10% Dextran sulfate, and washing twice for 30 minutes at 60°C in a wash solution comprising 0.1 X SSC and 1% SDS. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, et al. (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al., (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

#### **Host Cells and Vectors of the Invention**

According to another aspect of the invention, host cells are provided, including prokaryotic and eukaryotic cells, comprising a polynucleotide of the invention (or vector of the invention) in a manner which permits expression of the encoded S20 ribosomal polypeptide. Polynucleotides of the invention may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein coding region or a viral vector. Methods for introducing DNA into the host cell well known and routinely practiced in the art include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. Expression systems of the invention include bacterial, yeast, fungal, plant, insect, invertebrate, and mammalian cells systems.

Suitable host cells for expression of S20 ribosomal polypeptides include prokaryotes, yeast, and higher eukaryotic cells. Suitable prokaryotic hosts to be used for the expression of human *Staphylococcus aureus* Ribosomal Protein Gene, S20 include bacteria of the genera *Escherichia*, *Bacillus*, and *Salmonella*, as well as members of the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*.

The isolated nucleic acid molecules of the invention are preferably cloned into a vector designed for expression in prokaryotic cells, rather than into a vector designed for expression in eukaryotic cells. Prokaryotic cells are preferred for expression of genes obtained from prokaryotes because prokaryotic cells are more economical sources of protein production and because prokaryotic hosts grow to higher density and are typically grown in media which is less expensive than that used for the growth of eukaryotic hosts.

In the event a eukaryotic host were used the possibilities may include, but are not limited to, the following: insect cells, African green monkey kidney cells (COS cells), Chinese hamster ovary cells (CHO cells), human 293 cells, and murine 3T3 fibroblasts.

5 Expression vectors for use in prokaryotic hosts generally comprise one or more phenotypic selectable marker genes. Such genes generally encode, *e.g.*, a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors  
10 (LTI, Bethesda, MD), Bluescript vectors (Stratagene), and pQE vectors (Qiagen). A representative cloning and expression scheme is provided by the following example.

### Example 2

#### Isolation and Cloning of the S20 Coding Region

Two primers were designed for PCR. SEQ ID NO:9 (GTGTT ATCGATA

15 ATGGCAAATATCAAATCTGCAATTAAACG)

This sequence includes an overhang (GTGTT), a *ClaI* site, the start codon and the next 26 bases of the S20 ribosomal polypeptide gene and

SEQ ID NO:10 ( 5' GTGTTGGATCC TTA TTT ATT TGC AGT CAT TAA TTG TG). This sequence includes an overhang (GTGTT), a *BamH1* site, the stop codon  
20 and the next 23 bases of S20 *S. aureus* ribosomal protein.

Staphylococcus aureus genomic DNA was used as a template. The buffer (N808-0006) and AmpliTaq® (N8080-0101) were purchased from Perkin Elmer Cetus . The 10 mM dNTP mix was obtained from Gibco BRL (Gaithersburg, MD). The reaction mix was 5 µl of buffer, 1 µl of dNTP mix, 1 ng of each primer, 1 ng of genomic DNA  
25 and 0.5 µl (2.5 units) of ampliTaq in a final volume of 50 µl.

The program for PCR was 94°C for 10 minutes and then 40 cycles of 94°C for 1 minute, 57°C for 30 seconds, and 72°C for one minute. The final extension phase was at 72°C for 3 minutes and the reactions were allowed to stay at 4°C until they were removed from the thermocycler.

#### 30 Vector Construction and Expression

The PCR products were purified, digested with *ClaI* and *BamH1* and ligated to the expression vector pSR-Tac which contains *Cla I* and *BamHII* cloning sites. This vector contains a tac promoter, an AT rich synthetic ribosome binding site, two

transcription terminators designated T1 and sib3 upstream of the tac promoter and downstream of the cloned gene, respectively, an ampicillin resistance gene derived from pBR322, and a ColE1 origin of replication. The Cla I restriction site is located immediately downstream of the ribosome binding site and the BamHI site is  
5 immediately upstream of the sib3 terminator. While this particular vector worked quite well it is expected that other vectors used in *E.coli* heterologous protein expression would be equally suitable.

After transformation into *E. coli* strain Top10 F' *lacI*<sup>q</sup>, the colonies were screened by DNA mini prep and restriction digestion to find the desired constructs.  
10 The constructs were sequenced and transformed into *E. coli* strain K12s F' *lacI*<sup>q</sup> for expression studies.  
Cells harboring the construct pSRTac-S20 were grown in 50 ml LB with ampicillin at 37°C. The cultures were induced with 10<sup>-3</sup> M IPTG during the midlog phase of growth and allowed to express for 3 hours. Then the cells were collected, sonicated  
15 and examined using gel electrophoresis.

Half a milliliter of the sonicated expression cultures were centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected as the soluble fraction and the pellet (insoluble fraction) was suspended in 10 mM Tris-HCl pH 8.0. These samples were electrophoresed on 20% acrylamide with DATD crosslinker. The S20  
20 protein was expressed at moderate levels and observed to be in the soluble fraction.

#### Polypeptides of the Invention

Overexpression in eukaryotic and prokaryotic hosts as described above facilitates the isolation of S20 polypeptides. The invention therefore includes isolated S20 polypeptides as set out in SEQ ID NO:2 and variants and conservative  
25 amino acid substitutions therein including labeled and tagged polypeptides.

The invention includes S20 polypeptides which are "labeled". The term "labeled" is used herein to refer to the conjugating or covalent bonding of any suitable detectable group, including enzymes (e.g., horseradish peroxidase, beta - glucuronidase, alkaline phosphatase, and beta-D-galactosidase), fluorescent labels  
30 (e.g., fluorescein, luciferase), and radiolabels (e.g., <sup>14</sup>C, <sup>125</sup>I, <sup>3</sup>H, <sup>32</sup>P, and <sup>35</sup>S) to the compound being labeled. Techniques for labeling various compounds, including proteins, peptides, and antibodies, are well known. See, e.g., Morrison, Methods in Enzymology 32b, 103 (1974); Syvanen et al., J. Biol. Chem. 284, 3762 (1973);

Bolton and Hunter, Biochem. J. 133, 529 (1973). The termed labelled may also encompass a polypeptide which has covalently attached an amino acid tag as discussed below.

In addition, the S20 polypeptides of the invention may be indirectly labeled.

5. This involves the covalent addition of a moiety to the polypeptide and subsequent coupling of the added moiety to a label or labeled compound which exhibits specific binding to the added moiety. Possibilities for indirect labeling include biotinylation of the peptide followed by binding to avidin coupled to one of the above label groups. Another example would be incubating a radiolabeled antibody specific for a histidine tag with a S20 polypeptide comprising a polyhistidine tag. The net effect is to bind the radioactive antibody to the polypeptide because of the considerable affinity of the antibody for the tag.
- 10

The invention also embraces variants (or analogs) of the S20 protein. In one example, insertion variants are provided wherein one or more amino acid residues supplement a S20 amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the S20 protein amino acid sequence. Insertional variants with additional residues at either or both termini can include for example, fusion proteins and proteins including amino acid tags or labels. Insertion variants include S20 polypeptides wherein one or more amino acid residues are added to a S20 acid sequence, or to a biologically active fragment thereof.

- 15
- 20

Insertional variants therfore can also include fusion proteins wherein the amino and/or carboxy termini of S20 is fused to another polypeptide. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the influenza HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag -peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an alpha -tubulin epitope peptide [Skinner et al.,

- 25
- 30

J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397(1990)]. In addition, the S20 polypeptide can be tagged with enzymatic proteins such as peroxidase and alkaline phosphatase.

5       In another aspect, the invention provides deletion variants wherein one or more amino acid residues in a S20 polypeptide are removed. Deletions can be effected at one or both termini of the S20 polypeptide, or with removal of one or more residues within the S20 amino acid sequence. Deletion variants, therefore, include all fragments of the S20 polypeptide.

10      The invention also embraces polypeptide fragments of the sequence set out in SEQ ID NO: 2 wherein the fragments maintain biological (e.g., ligand binding or RNA binding and/or other biological activity) Fragments comprising at least 5, 10, 15, 20, 25, 30, 35, or 40 consecutive amino acids of SEQ ID NO: 2 are comprehended by the invention. Fragments of the invention having the desired 15 biological properties can be prepared by any of the methods well known and routinely practiced in the art.

The present invention also includes variants of the aforementioned polypeptide, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like 20 characteristics. Variant polypeptides include those wherein conservative substitutions have been introduced by modification of polynucleotides encoding polypeptides of the invention. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that 25 has similar properties. Exemplary conservative substitutions are set out in Table A (from WO 97/09433, page 10, published March 13, 1997 (PCT/GB96/02197, filed 9/6/96), immediately below.

**Table A**  
**Conservative Substitutions I**

30

SIDE CHAIN	CHARACTERISTIC	AMINO ACID
------------	----------------	------------

**Aliphatic**

Non-polar	G A P
Polar - uncharged	I L V
5 Polar - charged	C S T M
Aromatic	N Q
Other	D E
10	K R
	H F W Y
	N Q D E

Alternatively, conservative amino acids can be grouped as described in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77] as set out in Table B, immediately below

15 **Table B**  
**Conservative Substitutions II**

	<b>SIDE CHAIN</b>	
	<b>CHARACTERISTIC</b>	<b>AMINO ACID</b>
20	Non-polar (hydrophobic)	
	A. Aliphatic:	A L I V P
	B. Aromatic:	F W
	C. Sulfur-containing:	M
	D. Borderline:	G
25	Uncharged-polar	
	A. Hydroxyl:	S T Y
	B. Amides:	N Q
	C. Sulfhydryl:	C
	D. Borderline:	G
30	Positively Charged (Basic):	K R H
	Negatively Charged (Acidic):	D E

As still another alternative, exemplary conservative substitutions are set out in Table C, immediately below.

**Table C**  
**Conservative Substitutions III**

	<u>Original Residue</u>	<u>Exemplary Substitution</u>
	Ala (A)	Val, Leu, Ile
	Arg (R)	Lys, Gln, Asn
10	Asn (N)	Gln, His, Lys, Arg
	Asp (D)	Glu
	Cys (C)	Ser
	Gln (Q)	Asn
15	Glu (E)	Asp
	His (H)	Asn, Gln, Lys, Arg
	Ile (I)	Leu, Val, Met, Ala, Phe,
	Leu (L)	Ile, Val, Met, Ala, Phe
	Lys (K)	Arg, Gln, Asn
20	Met (M)	Leu, Phe, Ile
	Phe (F)	Leu, Val, Ile, Ala
	Pro (P)	Gly
	Ser (S)	Thr
	Thr (T)	Ser
25	Trp (W)	Tyr
	Tyr (Y)	Trp, Phe, Thr, Ser
	Val (V)	Ile, Leu, Met, Phe, Ala

Generally it is anticipated that the S20 polypeptide will be found primarily intracellularly, the intracellular material can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press,

homogenization, and/or sonication followed by centrifugation. The S20 polypeptide is found primarily in the supernatant after centrifugation of the cell homogenate, and the S20 polypeptide can be isolated by way of non-limiting example by any of the methods below.

5        In those situations where it is preferable to partially or completely isolate the S20 polypeptide, purification can be accomplished using standard methods well known to the skilled artisan. Such methods include, without limitation, separation by electrophoresis followed by electroelution, various types of chromatography (immunoaffinity, molecular sieve, and/or ion exchange),  
10 and/or high pressure liquid chromatography. In some cases, it may be preferable to use more than one of these methods for complete purification.

Purification of S20 polypeptide can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (S20/hexaHis) or other small peptide such as FLAG (Eastman  
15 Kodak Co., New Haven, Conn.) or myc (Invitrogen, Carlsbad, Calif.) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag or for the polypeptide directly (i.e., a monoclonal antibody specifically recognizing S20). For example, polyhistidine binds with great affinity and  
20 specificity to nickel, thus an affinity column of nickel (such as the Qiagen Registered TM nickel columns) can be used for purification of S20/polyHis. (See for example, Ausubel et al., eds., Current Protocols in Molecular Biology, Section 10.11.8, John Wiley & Sons, New York [1993]).

Even if the S20 polypeptide is prepared without a label or tag to facilitate  
25 purification. The S20 of the invention may be purified by immunoaffinity chromatography. To accomplish this, antibodies specific for the S20 polypeptide must be prepared by means well known in the art. Antibodies generated against the S20 polypeptides of the invention can be obtained by administering the polypeptides or epitope-bearing fragments, analogues or cells to an animal, preferably a  
30 nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., Nature 256: 495-497 (1975); Kozbor et al., Immunology Today

4: 72 (1983); Cole et al., pg. 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985).

Where the S20 polypeptide is prepared without a tag attached, and no  
5 antibodies are available, other well known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be  
10 combined to achieve increased purity. A representative purification scheme is detailed below.

### Example 3

#### Large Scale Purification of S20 Protein

S20-expressing *E. coli* cell paste resulting from 6 liters of fermentation was  
15 resuspended in ~ 70 mL Tris buffer pH 7.4 containing 1 mM MgCl<sub>2</sub> and 1 mM DTT. One Complete® EDTA-free protease inhibitor pellet (Boehringer Mannheim, Indianapolis, IN) was added to the suspended cells. The cells were lysed by passage three times through a French Press @ 10,000 PSI. A soluble fraction was prepared from the cellular lysate by ultracentrifugation @ 100,000 x g for 60 minutes @ 4° C.  
20 The soluble fraction was injected onto a HiPrep SP<sub>XL</sub> 16/10 cation exchange column which had been equilibrated in 50 mM Tris buffer pH 7.4, 1 mM MgCl<sub>2</sub>, and 1 mM DTT. The column flow rate was 4 mL/min. The column was washed with buffer until the Abs<sub>280</sub> of the column eluate was less than 0.01. Material was eluted off of the HiPrep SP<sub>XL</sub> column with a linear gradient of 0-700 mM NaCl in column buffer over  
25 20 column volumes. The column profile is shown in Figure 2. Fractions were collected and analyzed by SDS-PAGE using 4-12% Bis-Tris NuPage® gels (Novex, San Diego, CA) employing a MES buffer system. The gel is shown in figure 3. The gel legend is shown below.

Key to S20 Gel			
Lane	Sample	Lane	Sample
1	MW Standards	11	Fraction 32
2	Crude Lysate	12	MW Standards
3	Fraction 25	13	Fraction 33
4	Fraction 26	14	Fraction 34
5	Fraction 27	15	Fraction 35
6	Fraction 28	16	Fraction 36
7	Fraction 29	17	Fraction 37
8	Fraction 30	18	Fraction 38
9	Fraction 31	19	Fraction 39
10	MW Standards	20	MW Standards

S20-containing fractions were further analyzed by liquid chromatography electrospray mass spectrometry (LC/MS-ESI) performed on a Finnigan LC/Q instrument. The results of the LC/MS-ESI analysis yielded an average mass of 8064 amu which would correspond to a *des*<sup>9</sup> form of *S. aureus* ribosomal protein S20. The calculated average mass of the intact S20 is calculated to be 9021.46. The calculated average mass of the *des*<sup>9</sup> form of S20 is 8064.25. The sequence of *S. aureus* S20 is shown below. The *des*<sup>9</sup> form of the protein is highlighted in bold type

MANIKSAIKRVKTTEKAEARNSQKSAMRTAVKNAKTAVSNNADNKNELVSLAVKLVD  
10 KAAQSNLIIHSNKADRIKSQMLTANK

In addition to preparing and purifying S20 polypeptide using recombinant DNA techniques, the S20 polypeptides, fragments, and/or derivatives thereof may be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield et al., (J. Am. Chem. Soc., 85:2149 [1963]), Houghten et al. (Proc Natl Acad. Sci. USA, 82:5132 [1985]), and Stewart and Young (Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, Ill. [1984]). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized S20 polypeptides or fragments may be oxidized using methods set forth in these references to form disulfide bridges. The S20 polypeptides or fragments are expected to have biological activity comparable to S20 polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with recombinant or natural S20 polypeptide.

**Ribosomal Assembly Assays**

70S ribosome particles in *E.coli* consist of 31 core ribosomal "L" proteins and two rRNAs (5S and 23S) in the 50S subunit and 21 "S" proteins and a single 16S rRNA in the 30S subunit. These particles constitute the basic machinery for bacterial protein translation. It is postulated that the *Staphylococcus aureus* ribosome is assembled in fashion to ribosomes in *E.coli*. The present invention provides several methods to study the *S.aureus* 30S subunit assembly and methods to screen for inhibitors of the assembly process.

Assembly of the 30S ribosomal subunit is an ordered process both *in vivo* and *in vitro*. Nomura, M. and Held, W.A. (1974), Noller and Nomura (1987). It is now well known that the 21 proteins which comprise the the *E. coli* 30S subunit assemble onto the the 16S rRNA in an ordered fashion *in vitro*. *Id.* These proteins have been defined as primary or secondary binders, according to whether they bind to the 16S RNA independently of other proteins or not. Proteins that bind directly to 16S rRNA include S4, S7, S8, S15, S17 and S20. Secondary binding proteins include S3, S5, S9, S10, S12, S14, S16 and S19.

Producing and purifying the *S.aureus* ribosomal "S" proteins which are most critical for the formation of functional 30S subunits including those that bind directly to 16S rRNA (i.e., S4, S7, S8, S15, S17 and S20) "direct binding S-proteins" and critical proteins that integrate themselves into the ribosome by reliance on protein-protein and/or protein-RNA interactions (non-direct binding S- proteins)(S3, S5, S9, S10, S12, S14, S16 and S19) provides myriad choices in designing methods for testing inhibitors of ribosomal assembly.

**16S RNA binding assay for ribosomal protein S20**

Because S20 is a direct binding S protein it makes possible an assay in which S20 binding to 16S RNA may be measured directly. Such an assay involves the incubation of S20 polypeptide with 16S RNA, separation of bound from unbound S20 and measurement of that fraction of the S20 that remains bound to the RNA. By way of non-limiting example one can envision numerous ways in which the presence of unbound or bound S20 could be detected. The S20 might be radiolabeled in any of a number of means including but not limited to, labeling *in vitro* by chemical or enzymatic means or *vivo* by metabolically labeling cells expressing S20.

As discussed above commonly used radioactive isotopes used for the radiolabeling of peptides and proteins and nucleic acids include but are not limited to

<sup>3</sup>H, <sup>14</sup>C, <sup>35</sup>S, <sup>125</sup>I and <sup>32</sup>P. In addition, of course, if the S20 polypeptide or is tagged with an amino acid tag, as described above, the tag and the covalently attached S20 protein can be detected by means well known in the art. In addition, the S20 polypeptide or a polynucleotide can be tagged with enzymatic proteins such as

5 peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) which are capable of being monitored for change in fluorescence intensity, wavelength shift, or fluorescence polarization (FP) or fluorescent resonance energy transfer (FRET). Another method of labeling polypeptides and nucleic acids includes biotinylation of the peptide of the peptide or nucleic acid

10 followed by binding to avidin coupled to one of the above label groups or a solid support. In addition of course, such an assay is amenable to being performed with the 16S RNA (or a fragment thereof) being labeled with a radiolabel, a tag, or indirectly with a molecule such as biotin. The assay may be performed entirely in solution phase or it may be performed with either the 16S RNA or the 20S

15 polypeptide immobilized. A common means of immobilization is to attach biotin to the molecule of interest and immobilize it by contacting with a solid support to which avidin is bound. By way of non-limiting example, an assay in which the S20 polypeptide is immobilized on a solid support and is used to bind radiolabeled 16S RNA and an assay in which all components are free in solution are described below.

20

#### Example 4

##### 16S RNA -S20 binding assay

Because S20 is known to bind directly to 16S rRNA isolated S20 protein is an important reagent for developing a protein:RNA binding assay. The reagents for such a screen include S20 protein and labeled 16S RNA or a fragment of 16S RNA

25 capable of binding the S20 polypeptide. Depending on the format of the assay, the S20 polypeptide or the 16S RNA may be labeled by means of radiolabeling or with tags which make the RNA or polypeptide amenable to immobilization to a solid support.

30

##### Preparation of Starting Materials

###### Cloning of 16S Ribosomal RNA

The complete 16S-rRNA gene was identified in the HGS data base on contig 168268 by homology to the *B. subtilis* sequence. Five prime sequence of 5'-TTTATGGAGAGTTGATCCTGGC-3' and the 3' sequence of 5'-GCGGCTGGATCACCTCCTTCT-3' is used to amplify the entire 16S-rRNA gene

from *S. aureus* (Oligo Etc; Wilsonville, OR). The amplified gene is cloned into pT7Blue using Novagen's (Madison, WI) Perfectly Blunt Cloning Kit. DNA template is created by PCR using a primer that had the T7 promoter on the 5' end sequence of the 16S-rRNA gene (5'-TAATACGACTCACTATAGTTTATGGA-  
 5 GAGTTGATCCTGGC-3'). The length of the amplified 16S-rRNA fragment can be altered by the selection of the 3' primer. Whole 16S-rRNA as well as shorter segments could be used for screening of S20-16S-rRNA antagonists. The crystal structure has been solved for the 30S subunit (Brian T. Wimberly, et al Structure of the 30S ribosomal subunit. *Nature*. vol 407; p327-338, 2000). Helical pieces, H8,  
 10 H9, H11, and H44 create a pocket for the S20 protein to bind. These smaller helical pieces can be used for screen of S20 antagonist. Fragmented segments can be generated with the same T7 promoter as the whole 16S-rRNA was created and can also be labeled.

Helical RNAs	5'	3'
H-44 Nucleotide 1419-1502 SEQ ID NO:21	CACCAACGAGAGTTGTAAAC (SEQ ID NO:59)	CACCCCAATCATTGTCCCAC (SEQ ID NO:60)
H-7,8,9,10,& 11 Nucleotide 120-322 SEQ ID NO:21	CACGTGGATAACCTACCTA (SEQ ID NO:61)	GTGGCCGATCACCCCTCTCAGG (SEQ ID NO:62)

15

<sup>3</sup>H-UTP or <sup>35</sup>S-ATP can be used to label the RNA if labeled RNA is desired.

Resulting RNAs are characterized by electrophoresis on acrylamide-urea gels, and

RNA concentrations are determined by UV spectroscopy using A<sub>260</sub> unit = 40 ug/ml.

The entire S16 ribosomal RNA gene sequence has been reported (Genbank Accession

20 # X68417 also US Patent No. 5,843,669 Sequence # 160). The sequence of the gene is included in this document as SEQ ID NO:21

#### *Biotinylation of S20*

Purified S20 is biotinylated with the Pierce EZ-link Sulfo-NHS-LC-Biotinylation Kit (Pierce, Rockford, IL). Briefly, 401 µl of S20 (about 6.0 mg/ml), 64 µl of Sulfo-NHS-

25 LC-Biotin (10 mg/ml), and 598 µl of kit PBS buffer is allowed to react on ice for 2 hours. Excess biotin is removed by column desalting, dialysis or both. Desalting is performed by adding the product to a 10 ml desalting column that had been equilibrated with 30 ml of PBS buffer. The one milliliter sample is allowed to permeate the gel and 1 ml fractions is collected. Fractions are monitored by the Bio

Rad Protein Assay (Bio Rad, Hercules, CA). Dialysis is performed using a Pierce Slide-A-Lyzer 10K cassette (Pierce, Rockford, IL), under constant stirring for 16 hours at 4°C against 2 liters of 30 mM Phosphate buffer (pH 7.0), 400 mM NaCl.

*Multiscreen Assay and Scintillation Proximity Assay (SPA)*

5     The binding assay reported by Vartikar (1989) is modified as follows: S20 was diluted into TK buffer (350 mM KCl, 10 mM  $\beta$ -mecaptoethanol, 30 mM Tris [pH 7.6]) and incubated at 37°C for 30 minutes. Labelled RNA is renatured in buffer (350 mM KCl, 20 mM MgSO<sub>4</sub>, 10 mM  $\beta$ -mecaptoethanol, 30 mM Tris [pH 7.6]) at 40°C for 20 minutes. After renaturation, the S20 (30  $\mu$ l) and 16S-rRNA (20  $\mu$ l) is incubated at 10 room temperature for 10 minutes. A Multiscreen HA opaque 96 well filtration plate (Millipore; Bedford, MA) is first prewetted with 100  $\mu$ l of Dulbecco's PBS for 10 minutes and vacuumed to remove excess fluid. The S20-16S-rRNA complex is transferred to the Multiscreen plate, incubated for 5 minutes, vacuumed, air dried for 1 hour, and counted with 40  $\mu$ l of scintillation cocktail on a Topcount™ Microplate

15    Scintillation Counter. The SPA assay is run almost identical to the Multiscreen assay except that it utilized biotinylated S20 and streptavidin coated SPA beads (Amersham) in the final reaction. As before the S20 and 16S-rRNA is allowed to react for 10 minutes. Fifty  $\mu$ l of SPA beads (20 mg/ml) is added to the 50  $\mu$ l of S20:16S-rRNA complex in a Dynatech Microlite plate and counted in a Topcount™ Microplate

20    Scintillation Counter. Inhibition studies are conducted with 16S/23S-rRNA and MS2-mRNA purchased from Roche Molecular Biochemicals, Indianapolis, IN. To identify potential inhibitors of the 16S RNA-20S complex the assay is run in the presence and absence of potential inhibitors and the effect on binding is assessed.

**Simultaneous assay of S4, S7, S8, S15, S17 and S20 binding to 16S RNA:**

25    While the discussion above, illustrates an assay useful for the identification of inhibitors which directly disrupt the interaction between the S20 polypeptide and the 16S ribosomal RNA. It is recognized that the binding of the S20 polypeptide may, in part, be dependent on the interaction of other direct binding S-proteins binding in concert to the 16S ribosomal RNA. Such dependence may be the result of alterations 30 in the conformation of the 16S ribosomal RNA or

In another embodiment, all the direct binding S-proteins can be incubated with 16S RNA and the presence of bound or unbound S20 polypeptide determined. Indeed, the identity of all of the bound or unbound proteins can be determined. The

identity of a bound or unbound S protein can be determined, for instance by a suitable mass spectrometry technique, such as matrix-assisted laser desorption/ionization combined with time-of-flight mass analysis ( MALDI -TOF MS) or electrospray ionization mass spectrometry (ESI MS). See Jensen et al., 1977, Protein Analysis By 5 Mass Spectrometry, In Creighton (ed.), Protein Structure, A Practical Approach (Oxford University Press), Oxford, pp. 29-57; Patterson & Aebersold, 1995, Electrophoresis 16: 1791-1814; Figeys et al., 1996, Analyt. Chem. 68: 1822-1828 (each of which is incorporated herein by reference in its entirety). Preferably, a separation technique such as HPLC or capillary electrophoresis is directly or indirectly 10 coupled to the mass spectrometer. See Ducret et al., 1996, Electrophoresis 17: 866-876; Gevaert et al., 1996, Electrophoresis 17: 918-924; Clauser et al., 1995, Proc. Natl. Acad. Sci. USA 92: 5072-5076 (each of which is incorporated herein by reference in its entirety).

15

### Example 5

#### Assay of S20 with Other Direct Binding Proteins

This assay is used to test for direct RNA:protein assembly. The starting material proteins are preferably prepared by recombinant means and over-expression in a suitable host essentially as described in Examples 1, 2 and 3 for S20 with obvious 20 modifications to reflect the differing sequences of the proteins involved. The nucleotide sequences of cDNA's encoding *S. aureus* direct binding ribosomal proteins S4, S7, S8, S15 and S17 are presented in SEQ ID NOS:11, 13, 15, 17 and 19 respectively. Sequences encoding S4, S7, S8, S15, and S17 can be isolated by means of the polymerase chain reaction. Primers are selected such that entire coding region 25 is isolated. The complete amino acid sequences of S4, S7, S8, S15, and S17 polypeptides are presented in SEQ ID NOS:12, 14, 16, 18 and 20. Sequences encoding S4, S7, S8, S15, and S17 can be isolated by means of probing a genomic *Staphylococcus aureus* library with probes designed from SEQ ID NOS:11, 13, 15, 17 and 19 as well. The polymerase chain reaction would be a preferred method because 30 it generally allows the isolation of a complete coding sequence in one experiment.

Methods for preparing and using probes and primers are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring

Harbor, N.Y., 1989; Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1987 (with periodic updates); and Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990.

5        Primers are selected to have low self- or cross-complementarity, particularly at the 3' ends of the sequence. Long homopolymer tracts and high GC content are avoided to reduce spurious primer extension. Primers are typically about 20 residues in length, but this length can be modified as well-known in the art, in view of the particular sequence to be amplified. Computer programs are available to aid  
10      in these aspects of the design. One widely used computer program for designing PCR primers is (OLIGO 4.0 by National Biosciences, Inc., 3650 Annapolis Lane, Plymouth, Mich.). Another is Primer (Version 0.5,(c) 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.).

Isolated 16S RNA is prepared as described in Example 4.

15      In this assay all six of the S-proteins that bind directly to 16S RNA are added together with test compound. Unbound S-proteins are then removed by size-separation or filtration. Automated LC/ESI ion-trap or MALDI-tof-MS is then used to determine if a particular S-protein is inhibited in its binding to 16S RNA. Mass spectrometry is an ideal detection tool since all of the S-protein average masses are  
20      known and unique. An example illustrates how specific inhibition of S20 protein binding to RNA is detected. The concept is illustrated in Figure 4.

RNA:protein assembly is assayed in 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 330 mM NaCl at 42 °C. The procedure is based on the conditions of Culver and Noller (RNA, 1999, 5: 832-843) except that 0.01% Nikkol detergent is removed  
25      because it significantly complicates the LC/MS analysis. Primary ribosomal binding proteins S4, S7, S8, S15, S17, and S20 are dialyzed overnight against 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 1 M NaCl. In the reconstitution, 200 pmol *in vitro* transcribed 16S RNA is incubated at 42 °C for 15 minutes. Then, 800 pmol S7, S8, S15, S17, and S4 each are added to the RNA, followed by 400 pmol S20. The NaCl concentration is then adjusted to 330 mM by adding 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>. The mixture is then incubated at 42 °C for 20 more minutes. The protein:RNA complex is then separated from the free proteins by spinning in a YM-30 Microcon at 500 xg for 20 minutes. The RNA is precipitated from the retentate

by adding 2 volumes of acetic acid and incubating on ice for 45 minutes. Proteins from both the flow-through and retentate are analyzed by LC/ESI ion trap mass spectrometry. The proteins are first separated on a C4 reversed phase column (Vydac) using a gradient from 98% of 0.1% TFA, 2% of 90% acetonitrile/0.1% TFA to 100% of 90% acetonitrile/0.1 % TFA. The intact mass of each protein are observed by electrospray mass spectrometry as it eluted from the column.

We have also been able to identify S20 in a mixture of primary ribosomal binding proteins by MALDI-TOF mass spectrometry. The mixture of proteins is passed over a C18 zip-tip (Millipore) to remove salts, eluting in 80% acetonitrile/0.1% TFA. A saturated solution of sinapinic acid is prepared in 30% acetonitrile/0.1% TFA. One microliter of the protein solution is mixed with ten microliters of the matrix solution, and 0.5 uL is spotted onto the stainless steel MALDI target. MALDI-TOF data were collected in linear mode from 6000-25000 Da, and the intact mass for S20 is observed.

15 Of course, purified direct binding proteins make possible assays to access the association of any or all direct binding proteins with 16S RNA. The invention of course, includes methods for testing for inhibitors of ribosomal assembly in which the incorporation of any direct binding protein into the polyribonucleotide protein complex is accessed.

20

### Example 6

#### Scintillation Proximity Assay (SPA)

##### Assay of S20 with Other Direct Binding Proteins

As in the previous example all S4, S7, S8, S15 and S17 are incubated together with 16S RNA followed by S20 ribosomal polypeptide in the presence and absence of 25 a test compound. Starting materials are prepared roughly as described in previous examples. In this example the 16S ribosomal RNA is end labeled with biotin and the S20 ribosomal polypeptide is radioactively labeled.

Primary ribosomal binding proteins S4, S7, S8, S15, S17, and S20 are dialyzed overnight against 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 1 M NaCl. In the 30 reconstitution, 200 pmol *in vitro* transcribed 16S RNA is incubated at 42 °C for 15 minutes. Then, 800 pmol S7, S8, S15, S17, and S4 each are added to the RNA, followed by 400 pmol S20. The NaCl concentration is then adjusted to 330 mM by adding 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>. Fifty µl streptavidin coated SPA

beads (20 mg/ml) is added to the 50  $\mu$ l of of the reaction mixture in a Dynatech Microlite plate and counted in a Topcount<sup>TM</sup> Microplate Scintillation Counter. To identify potential inhibitors of S20 incorporation into the polyribonucleotidprotein complex, the assay is run in the presence and absence of potential inhibitors and the 5 effect on binding is assessed.

#### Protein-protein Interaction Assembly Screen

The isolated S20 polypeptide of the invention also makes possible an assay through which one may detect all possible protein-protein disruptions in the 30S assembly process. This is important since published assembly maps are not based on 10 the myriad of possible protein-protein interactions that may occur. In practice these maps are based on limited S-protein combinations that were tested *in vitro*. This assay makes use of the fact that the assembly of ribosomes in general and the 30S subunit in particular, is an ordered process and makes use of all 21 small subunit ribosomal proteins or a limited subset of those proteins. The S3 ribosomal protein is known to 15 integrate itself last or very late in the ribosomal assembly process. Its efficient integration is known to be dependent upon the proper integration of the direct binding ribosomal proteins as well non-direct binding proteins. Proper partial assembly is monitored by measuring the incorporation of S3 ribosomal polypeptide into the partially or fully assembled ribosome. In the alternative, improper or disrupted 20 assembly can be assayed by exclusion of S3 ribosomal polypeptide from the ribosome

The S3 ribosomal protein may be labeled as discussed hereinbefore for ease of detection. The 16S ribosomal RNA or a direct binding ribosomal peptide may immobilized or the entire assay may be performed with all components in solution phase. The starting materials for the assays are preferably prepared by recombinant 25 means. The DNA sequences encoding all 21 30S subunit proteins are provided in the sequence listings as well as the amino acids sequences encoded by each. The invention provides ribosomal assembly assays utilizing all 21 small subunit ribosomal proteins as well as a select subset of proteins readily apparent to one skilled in the art.. Sequences encoding each protein can be isolated by means of the 30 polymerase chain reaction. Primers are selected as discussed previously. Primers are selected as discussed previously. Primers are selected such that entire coding region is isolated. Methods for preparing and using probes and primers are discussed above.

Exemplary forward and reverse primers suitable for amplification of S4, S6, and S18 are described listed here by way of example. One skilled in the art would recognize that other primers may be equally suitable.

S4 Forward 5'-TATATTATCGATAATGGCTCGATTAGAGGT-3' (SEQ ID NO:53)

S4 Reverse 5'-TATAGGATCCTAACGGATTAATTGTTCTCGTTAATT-3' (SEQ ID NO:54)

S18 Forward 5'-TATATTATCGATAATGGCAGGTGGACCAAGAAG-3' (SEQ ID NO:55)

10 S18 Reverse 5'-TATAGGATCCTTATTGTTCTCTTAAACAT-3' (SEQ ID NO:56)

S6 Forward 5'-TATATTATCGATAATGAAGAACATATGAAGTTAT-3' (SEQ ID NO:57)

S6 Reverse 5'-TATAGGATCCTTACTTGTCTCGTCTTCAC-3' (SEQ ID NO:58)

The following is provided by way of non-limiting example.

15 **Example 7**

**Partial Ribosomal Assembly Assay**

In this assay format several S-proteins are allowed to interact with 16S RNA in the presence of a test compound (Fig.5). The assay makes use of all of the direct binding ribosomal proteins except S15 (S4, S7, S8, S17 and S20) and a select group 20 of *S. aureus* ribosomal proteins which integrate themselves into the ribosome by reliance on protein-protein or protein-RNA interactions (S3, S5, S9, S10, S12, S14, S16 and S19)

The starting material proteins are prepared by recombinant means and over-expression in a suitable host essentially as described in Examples 1, 2 and 3 for the 25 S20 polypeptide of the invention with obvious modifications to reflect the differing sequences of the proteins involved. The nucleotide sequences of cDNA's encoding *S. aureus* direct binding ribosomal proteins S4, S7, S8, and S17 are presented in SEQ ID NOS:11, 13, 15, and 19 respectively. The production of the isolated S20 polypeptide of the invention is described hereinbefore.

30 The nucleotide sequences of cDNA's encoding *S. aureus* ribosomal proteins which integrate themselves into the ribosome by reliance on protein-protein or protein-RNA interactions (non-direct binding ribosomal proteins) S3, S5, S9, S10, S12, S14, S16 and S19 are presented in SEQ ID NOS: 26, 28, 32, 34, 38, 42, 44, and

48 respectively. Nucleotide sequences encoding *S. aureus*. S3, S4, S5, S7, S8, S9, S10, S12, S14, S16 S17 and S19 can be isolated by means of the polymerase chain reaction. Primers are selected such that the entire amino acid coding region is isolated. The complete amino acid sequences of *S. aureus* S3, S4, S5, S7, S8, S9, 5 S10, S12, S14, S16 S17 and S19 polypeptides are presented in SEQ ID NOS:27, 12, 29, 14, 16, 33, 35, 39, 43, 45, 20 and 49. Sequences encoding S3, S4, S5, S7, S8, S9, S10, S12, S14, S16 S17 and S19 can be isolated by means of probing a genomic *Staphylococcus aureus* library with probes designed from SEQ ID NOS:12, 28, 13, 15, 32, 34, 38, 42, 44, 19, and 48 as well. The polymerase chain reaction would be a 10 preferred method because it generally allows the isolation of a complete coding sequence in one experiment. The S3 protein is labeled, preferably radiolabeled.

RNA:protein assembly is assayed in 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 330 mM NaCl at 42 °C. The procedure is based on the conditions of Culver and Noller (RNA, 1999, 5: 832-843) except that 0.01% Nikkol detergent is removed 15 because it significantly complicates the LC/MS analysis. Ribosomal proteins S3, S4, S5, S7, S8, S9, S10, S12, S14, S16, S17, S19 and S20 are dialyzed overnight against 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 1 M NaCl. In the reconstitution, 200 pmol *in vitro* transcribed 16S RNA is incubated at 42 °C for 15 minutes. Then, 800 pmol ribosomal proteins S4, S7, S8, S17, and S20 added to the RNA, followed by 20 ribosomal proteins, S5, S9, S10, S12, S14, S16 and S19. The NaCl concentration is then adjusted to 330 mM by adding 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>. The mixture is then incubated at 42 °C for 20 more minutes. 800 pmol labeled ribosomal protein S3 is then added.

Unbound S-proteins are removed by size-separation or filtration. If the labelled S3 25 protein is present in the RNA:multiprotein complex then the compound does not inhibit any specific protein-protein interactions during the assembly process. If the compound prevents the incorporation of labelled S3 protein then the assay reveals that the test compound inhibits a protein-protein interaction.

The partially assembled RNA:multiprotein complex is then analyzed by 30 LC/ion-trap electrospray analysis to determine the S-protein components in the partially assembled complex. Alternatively MALDI-tof-MS can be used. Knowing the identity of S-proteins in the partially assembled complex and published knowledge of how the 30S subunit is assembled *in vitro* (Noller and Nomura (1987) the protein-

protein interaction that is disrupted by the test compound may be determined. The exact protein-protein interaction that is disrupted can be determined using selective combinations of S-proteins added to 16S RNA and compound. As stated above, this is an important confirmation process since published *in vitro* assembly maps are based  
5 on a limited data set. Assembly disruption by the test compound can be independently verified by analytical ultracentrifugation analysis (Fig.6). In this process the partially assembled 30S complex is differentiated from intact complex by displaying a lower rate of sedimentation in a given centrifugal field (i.e., as measured by a lower sedimentation constant, expressed in Svedberg units or S). The contents of  
10 sedimentation clusters can be verified by mass spectrometry.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

15 The entire disclosure of all publications cited herein are hereby incorporated by reference.

**CLAIMS**

What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence that encodes an amino acid sequence having at least 85% identity with SEQ ID NO:2
- 5
2. An isolated nucleic acid comprising the nucleotide sequence having least 85% identity with SEQ ID NO:1
- 10
3. An isolated nucleic acid comprising a nucleotide sequence that encodes the amino acid sequence of SEQ ID NO:2
4. An isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO:1
- 15
5. An isolated nucleic acid comprising a nucleotide sequence that encodes the amino acid sequence having at least 85% identity with residues 10 through 83 of SEQ ID NO:2
- 20
6. An isolated nucleic acid comprising the nucleotide sequence having least 85% identity with nucleotides 28 through 249 of SEQ ID NO:1
- 25
7. An isolated nucleic acid comprising a nucleotide sequence that encodes the amino acid sequence residues 10 through 83 of SEQ ID NO:2
8. An isolated nucleic acid comprising nucleotides 28 through 249 of SEQ ID NO:1
- 20
9. An isolated S20 ribosomal polypeptide comprising an amino acid sequence having least 85% identity to the sequence of SEQ ID NO:2.
- 30
10. An isolated S20 ribosomal polypeptide comprising the amino acid sequence of SEQ ID NO:2.

11. An isolated S20 ribosomal polypeptide comprising an amino acid sequence having least 85% identity to residues 10 through 83 of SEQ ID NO:2.
12. An isolated S20 ribosomal polypeptide comprising residues 10 through 83 of SEQ  
5 ID NO:2
13. The isolated S20 ribosomal polypeptide of claim 11 which comprises a label.
14. The isolated S20 ribosomal polypeptide of claim 11 wherein the label is selected  
10 from the group consisting of: radiolabels, fluorescent labels, amino acid tags and biotin.
15. The isolated S20 ribosomal polypeptide of claim 13 wherein said S20 ribosomal  
15 polypeptide comprises a radiolabel.
16. The isolated S20 ribosomal polypeptide of claim 13 wherein said S20 ribosomal  
polypeptide comprises a fluorescent label.
17. The isolated S20 ribosomal polypeptide of claim 13 wherein said S20 ribosomal  
20 polypeptide comprises an amino acid tag.
18. The isolated S20 ribosomal polypeptide of claim 13 wherein said S20 ribosomal  
polypeptide comprises a biotin molecule
- 25 19. A vector comprising the nucleic acid of claim 5
20. A host cell comprising the vector of claim 19
21. A method of making isolated an S20 ribosomal polypeptide comprising:  
30 a) introducing the nucleic acid of claim 5 into a host cell  
b) maintaining said host cell under conditions whereby said nucleic acid is expressed to produce said S20 ribosomal polypeptide  
c) purifying said S20 ribosomal polypeptide

22. A method for testing for inhibitors of ribosomal assembly comprising the steps of:

a) contacting the S20 ribosomal polypeptide of claim 11 with a 16S ribosomal RNA

i 5 (i) in the presence of a test agent; and  
(ii) in the absence of said test agent; and

b) determining the amount of said S20 ribosomal polypeptide specifically bound to said RNA

10 (i) in the presence of a test agent; and  
(ii) in the absence of said test agent; and

c) comparing the amount of said S20 ribosomal polypeptide determined in step (b)(i) to the amount of said S20 ribosomal polypeptide determined in step (b)(ii);

23. The method of claim 22 wherein said S20 ribosomal polypeptide comprises

15 residues 10 through 83 of SEQ ID NO:2

24. The method of claim 22 wherein said S20 ribosomal polypeptide is labeled

25. The method of claim 22 wherein said S20 ribosomal polypeptide comprises a  
20 radiolabel

26. The method of claim 22 wherein said S20 ribosomal polypeptide comprises an amino acid tag.

25 27. The method of claim 22 wherein said S20 ribosomal polypeptide comprises a biotin molecule.

28. The method of claim 22 wherein said 16S ribosomal RNA comprises nucleotide position 1419 to 1502 of SEQ ID NO:21.

30 29. The method of claim 22 wherein said 16S ribosomal RNA comprises nucleotide position 120 to 322 of SEQ ID NO:21.

30. The method of claim 22 wherein said 16S ribosomal RNA is labeled
31. The method of claim 22 wherein said 16S ribosomal RNA comprises a radiolabel
- 5 32. The method of claim 22 wherein said 16S ribosomal RNA comprises a biotin molecule
33. The method of claim 22 wherein said S20 ribosomal polypeptide is attached to a solid support.

10

34. The method of claim 22 wherein said 16S ribosomal RNA is attached to a solid support
35. A method for testing for inhibitors of ribosomal assembly comprising the steps of:
  - 15 Contacting at least one direct binding ribosomal polypeptide selected from the group consisting of S4, S7, S8, S15, S17 and S20 with 16S ribosomal RNA
    - (i) in the presence of a test agent; and
    - (ii) in the absence of said test agent; and
  - b) determining the amount of direct binding protein bound to the RNA
    - 20 (i) in the presence of a test agent; and
    - (ii) in the absence of said test agent; and
  - c) comparing the amount direct binding protein determined in step (b)(i) to the amount of direct binding protein determined in step (b)(ii);
- 25 36. The method of claim 35 wherein the direct binding ribosomal proteins comprise S4, S7, S8 and S20.
37. The method of claim 35 wherein the direct binding ribosomal proteins comprise S4, S7, S8, S17 and S20

30

38. The method of claim 35 wherein the direct binding ribosomal proteins comprise S4, S7, S8, S17, S15 and S20.

39. The method of claim 35 wherein said direct binding ribosomal polypeptide is labeled
40. The method of claim 35 wherein said direct binding ribosomal polypeptide comprises a radiolabel
41. The method of claim 35 wherein said direct binding ribosomal polypeptide comprises an amino acid tag.

10 42. The method of claim 35 wherein said direct binding ribosomal polypeptide comprises a biotin molecule

43. The method of claim 35 wherein said 16S ribosomal RNA is labeled
- 15 44. The method of claim 35 wherein said 16S ribosomal RNA comprises a radiolabel
45. The method of claim 35 wherein said 16S ribosomal RNA comprises a biotin molecule

20 46. The method of claim 35 wherein said direct binding ribosomal polypeptide is attached to a solid support.

47. The method of claim 35 wherein said 16S ribosomal RNA is attached to a solid support

25

48. A method for testing for inhibitors of ribosomal assembly comprising the steps of:

- a) contacting S20 ribosomal polypeptide and at least one other direct binding ribosomal polypeptide selected from the group consisting of S4, S7, S8, S15 and S17 with 16S ribosomal RNA in the
  - (i) in the presence of a test agent; and
  - (ii) in the absence of said test agent; and

b) determining the amount of S20 ribosomal polypeptide or any other direct binding protein bound to the RNA

(i) in the presence of a test agent; and

(ii) in the absence of said test agent; and

5 c) comparing the amount of S20 ribosomal polypeptide or any other direct binding protein determined in step (b)(i) to the amount of S20 ribosomal polypeptide or any other direct binding protein determined in step (b)(ii);

10 49. The method of claim 48 wherein the other direct binding ribosomal proteins comprise S4, S7, S8.

50. The method of claim 48 wherein the other direct binding ribosomal proteins comprise S4, S7, S8 and S17.

15 51. The method of claim 48 wherein the other direct binding ribosomal proteins comprise S4, S7, S8, S17, S15.

52. The method of claim 48 wherein said S20 ribosomal polypeptide or other direct binding ribosomal polypeptide is labeled

20 53. The method of claim 48 wherein said S20 ribosomal polypeptide or other direct binding ribosomal polypeptide comprises a radiolabel

54. The method of claim 48 wherein said S20 ribosomal polypeptide or other direct binding ribosomal polypeptide comprises an amino acid tag.

25 55. The method of claim 48 wherein said S20 ribosomal polypeptide or other direct binding ribosomal polypeptide comprises a biotin molecule

30 56. The method of claim 48 wherein said 16S ribosomal RNA is labeled

57. The method of claim 48 wherein said 16S ribosomal RNA comprises a radiolabel

58. The method of claim 48 wherein said 16S ribosomal RNA comprises a biotin molecule

59. The method of claim 48 wherein said S20 ribosomal polypeptide or other direct binding ribosomal polypeptide is attached to a solid support.

60. The method of claim 48 wherein said 16S ribosomal RNA is attached to a solid support

10 61. A method for testing for inhibitors of ribosomal assembly comprising the steps of:

a.) contacting at least one direct binding ribosomal polypeptide selected from the group consisting of S4, S7, S8, S15, S17 and S20 with 16S ribosomal RNA to form a polyribonucleotide protein complex and;

15 b) contacting said polyribonucleotide protein complex with at least one non-direct binding ribosomal polypeptide selected from the group consisting of S1, S2, S3, S5, S6, S9, S10, S11, S12, S13, S14, S16, S18, S19, and S21.

(i) in the presence of a test agent; and

(ii) in the absence of said test agent; and

20 c) determining the amount of at least one non- direct binding ribosomal polypeptide bound to the RNA

(i) in the presence of a test agent; and

(ii) in the absence of said test agent; and

25 d.)comparing the amount of least one non direct binding ribosomal polypeptide determined in step (c)(i) to the amount of non-direct binding ribosomal polypeptide protein determined in step (c)(ii);

62. The method of claim 61 wherein the direct binding ribosomal proteins comprise S4, S7, S8.

30

63. The method of claim 61 wherein the direct binding ribosomal proteins comprise S4, S7, S8 and S17.

64. The method of claim 61 wherein the direct binding ribosomal proteins comprise S4, S7, S8, S17, S15.

5        65. The method of claim 61 wherein the direct binding ribosomal proteins comprise S4, S7, S8, S17, S15 and S20

66. The method of claim 61 wherein the non-direct binding ribosomal proteins comprise S16

10      67. The method of claim 61 wherein the non-direct binding ribosomal proteins comprise S3, S5, S9, S10, S12, S14, S16 and S19

68. The method of claim 61 wherein said direct binding or non-direct binding ribosomal polypeptide is labeled

15      69. The method of claim 61 wherein said direct binding or non-direct binding ribosomal polypeptide comprises a radiolabel

70. The method of claim 61 wherein said direct binding or non-direct binding ribosomal polypeptide comprises an amino acid tag.

20      71. The method of claim 61 wherein said direct binding or non-direct binding ribosomal polypeptide comprises a biotin molecule

72. The method of claim 61 wherein said 16S ribosomal RNA is labeled

25      73. The method of claim 61 wherein said 16S ribosomal RNA comprises a radiolabel

74. The method of claim 61 wherein said 16S ribosomal RNA comprises a biotin molecule

30      75. The method of claim 61 wherein said direct binding or non-direct binding ribosomal polypeptide is attached to a solid support.

76. The method of claim 61 wherein said 16S ribosomal RNA is attached to a solid support

77. A method for testing for inhibitors of ribosomal assembly comprising the steps of:

5       a.) contacting S4, S7, S8, S17 and S20 ribosomal polypeptides with 16S ribosomal RNA to form a polyribonucleotide protein complex and;

      b) contacting said polyribonucleotide protein complex with non-direct binding ribosomal polypeptides S3, S5, S9, S10, S12, S14, S16 and S19 to form a resultant polyribonucleotide protein complex

10       (iii)     in the presence of a test agent; and

            (iv)    in the absence of said test agent; and

      d) contacting non-direct binding ribosomal polypeptide S3 with said resultant polyribonucleotide protein complex;

and determining the amount of said non-direct binding ribosomal polypeptide S3 bound to said resultant polyribonucleotide protein complex;

15       (i)       formed in the presence of said test agent; and

            (ii)    formed in the absence of said test agent; and

      e) comparing the amount of S3 determined in step (d)(i) to the amount of S3 determined in step (d)(ii)

20       78. The method of claim 77 wherein said non-direct binding ribosomal polypeptide S3 is labeled.

79. The method of claim 78 wherein said non-direct binding ribosomal polypeptide S3 is radiolabeled

*Figure 1*

ATGGCAAATACTGCAATTAAACGTGTAAAACAAACTGAAAAAGCTGAAGCACCGC<sup>60</sup>

M A N I K S A I K R V K T T E K A E A R

AACATTTCACAAAAGAGTGGCAATGGCGTACAGGCAGTTAAAACGGCTAAACAGCTGTTCA<sup>120</sup>

N I S Q K S A M R T A V K N A K T A V S

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N N A D N K N E L V S L A V K L V D K A

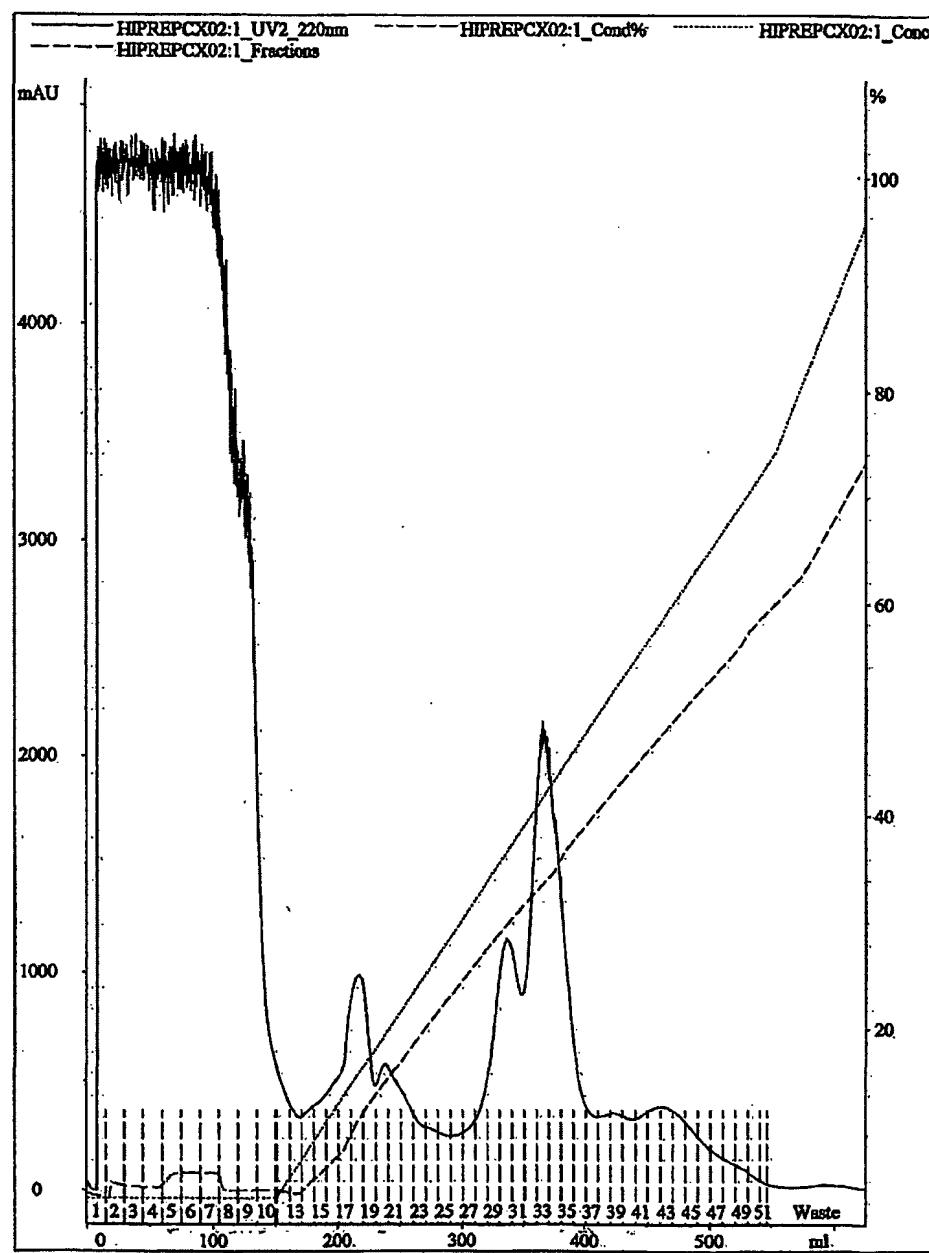
GCTCAAAGTAATTAAATACATTCAAAACAAAGCTGACCGTTATTAAATCACAAATTAAATGACT<sup>240</sup>

A Q S N L I H S N K A D R I K S Q L M T

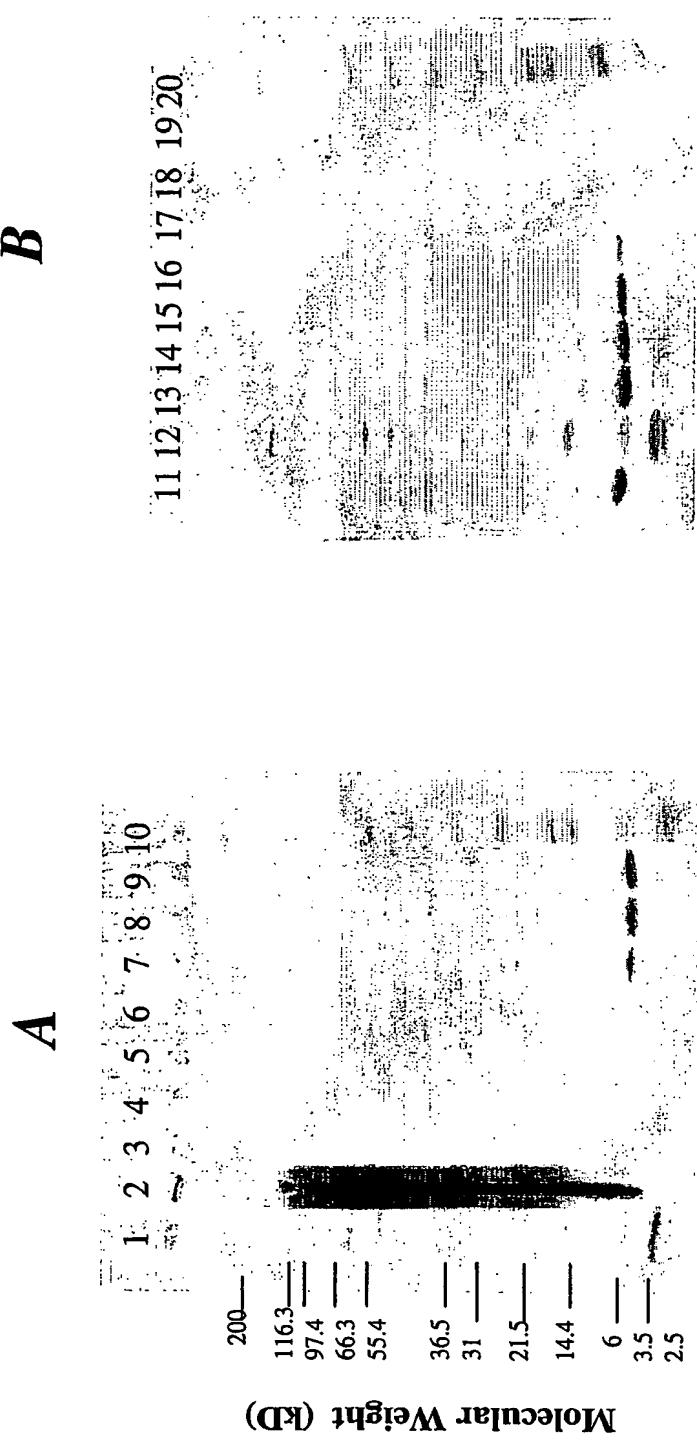
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A N K \*

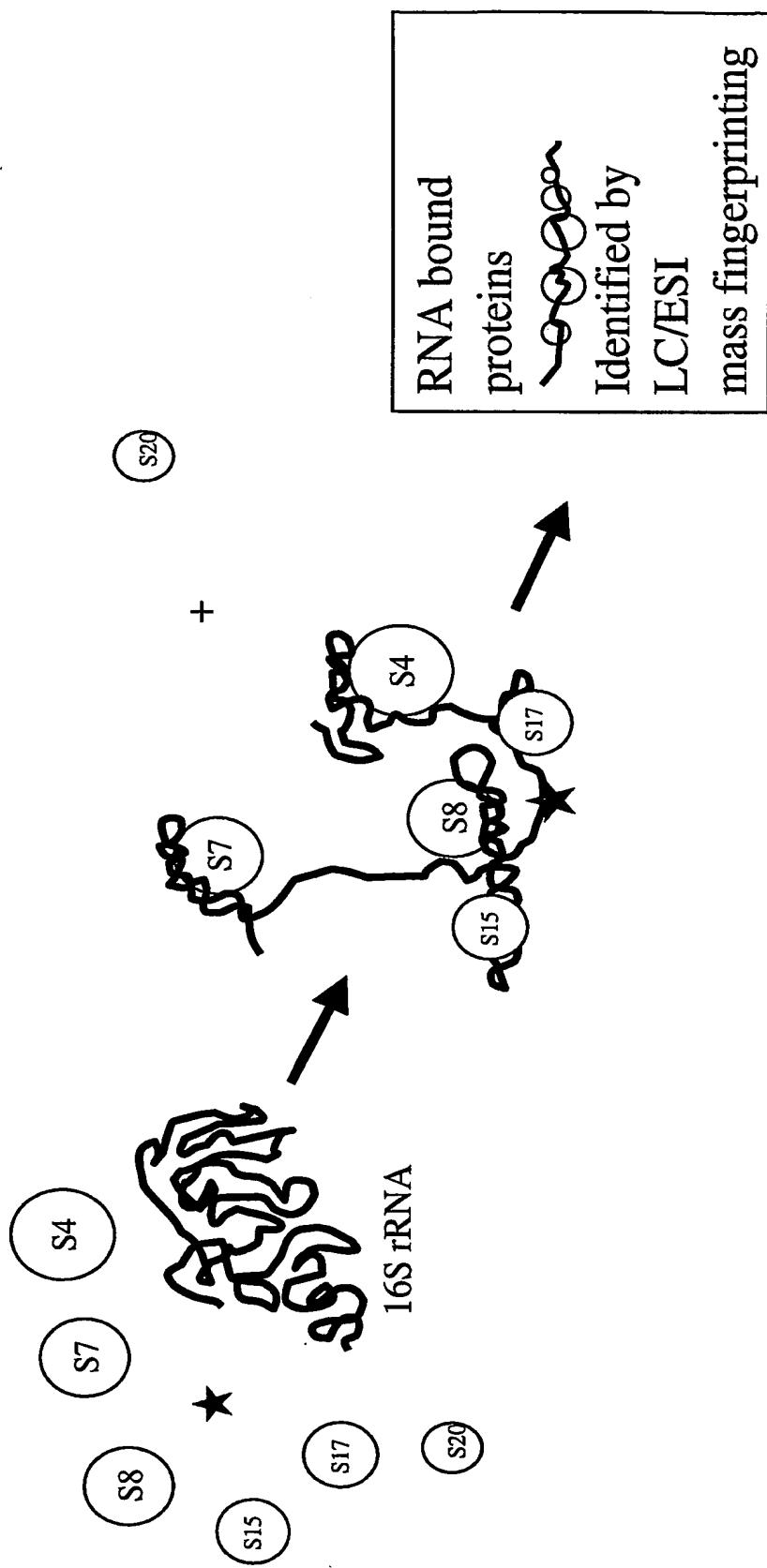
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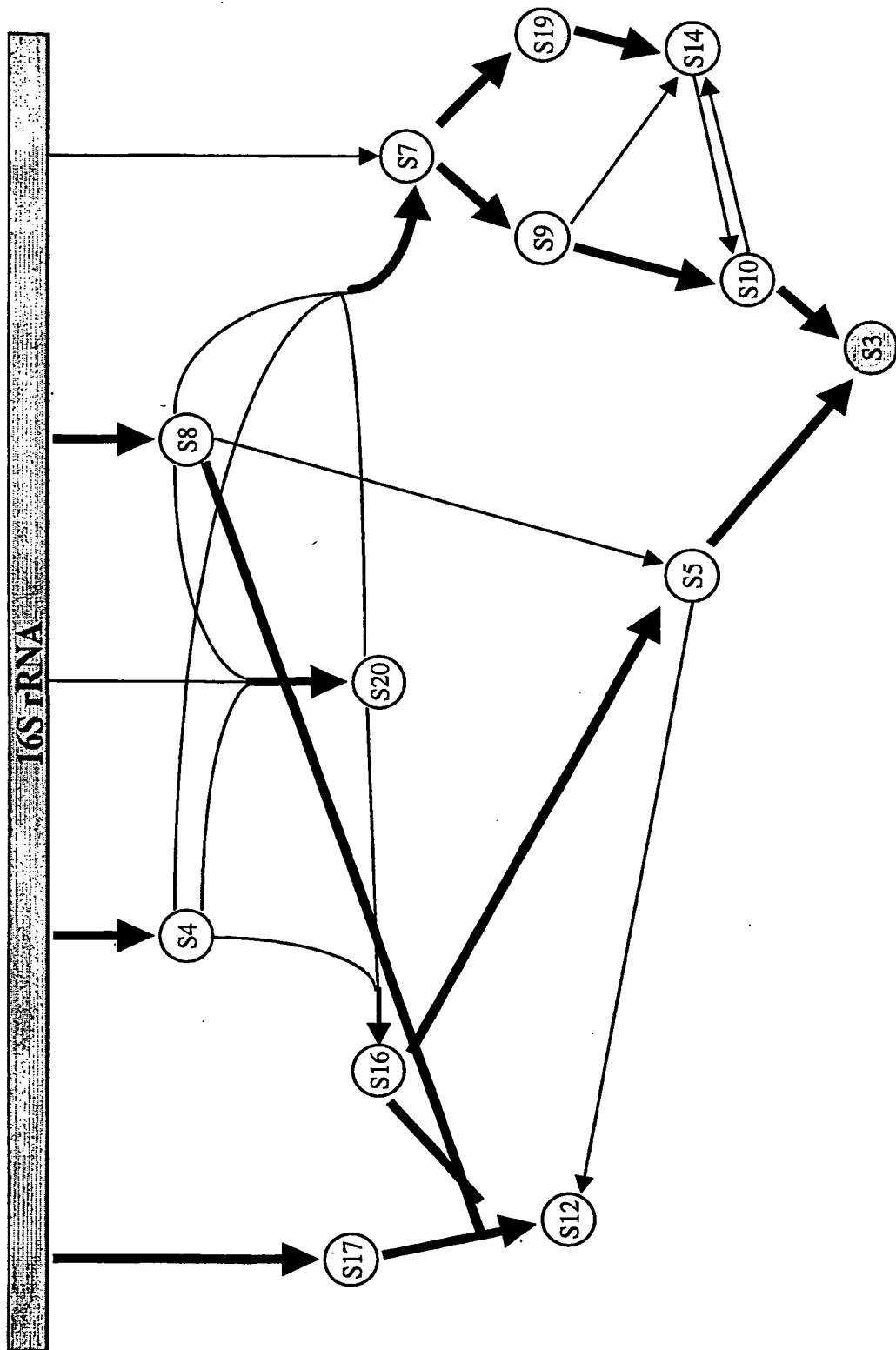
*Figure 3*



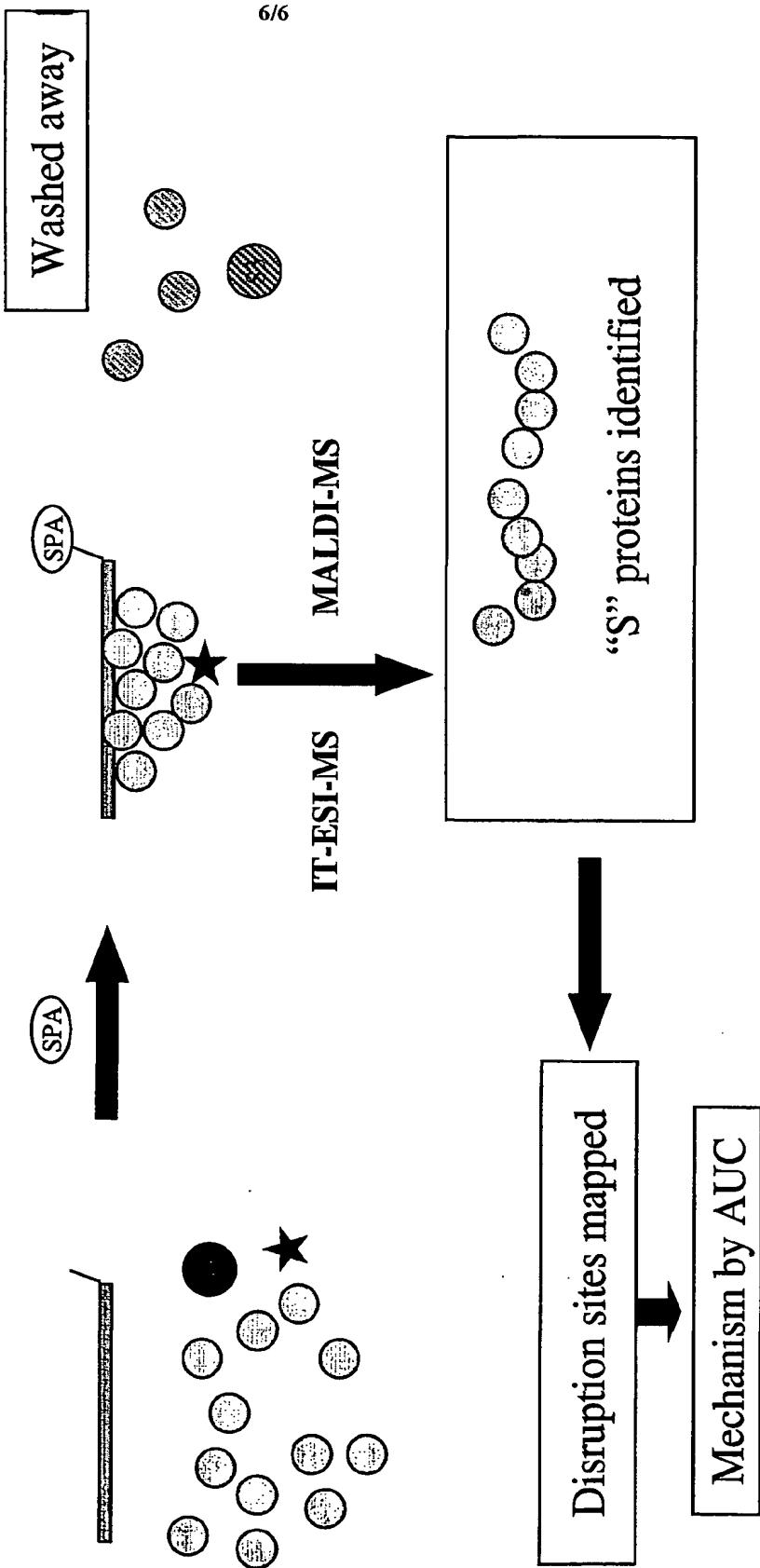
*Figure 4*



*Figure 5*



# Figure 6



## SEQUENCE LISTING

<110> Pearson, James D  
Slightom, Jerry  
Chosay, John G.  
McCroskey, Mark C.  
Shinabarger, Dean L.  
Wilcox, Sheri

<120> Complete Nucleotide Sequence of *Staphylococcus aureus*  
Ribosomal Protein Gene, S20 and Methods for the  
Identification of Antibacterial Substances

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aataacgctg ataataaaaaa tgaatttagta agcttagcag ttaagtttgt agacaaagct 180  
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20 25 30

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35 40 45

Leu Val Ser Leu Ala Val Lys Leu Val Asp Lys Ala Ala Gln Ser Asn  
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actggtaaag aatttagaaaa acgtccttac gcaccaggac aacatggtcc aaaccaacgt 180  
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				20				25				30			

Pro	Gly	Gln	His	Gly	Pro	Asn	Gln	Arg	Lys	Lys	Leu	Ser	Glu	Tyr	Gly
					35			40				45			

Leu	Gln	Leu	Arg	Glu	Lys	Gln	Lys	Leu	Arg	Tyr	Leu	Tyr	Gly	Met	Thr
					50			55			60				

Glu	Arg	Gln	Phe	Arg	Asn	Thr	Phe	Asp	Ile	Ala	Gly	Lys	Lys	Phe	Gly
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Val	His	Gly	Glu	Asn	Phe	Met	Ile	Leu	Leu	Ala	Ser	Arg	Leu	Asp	Ala
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Val	Val	Tyr	Ser	Leu	Gly	Leu	Ala	Arg	Thr	Arg	Arg	Gln	Ala	Arg	Gln
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Leu	Val	Asn	His	Gly	His	Ile	Leu	Val	Asp	Gly	Lys	Arg	Val	Asp	Ile
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Pro	Ser	Tyr	Ser	Val	Lys	Pro	Gly	Gln	Thr	Ile	Ser	Val	Arg	Glu	Lys
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Leu	Ile	Arg	Glu	Tyr	Tyr	Ser	Arg								
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35 40 45

Leu Val Glu Gln Arg Ser Gly Arg Asp Ala Leu Glu Val Phe Glu Glu  
50 55 60

Ala Ile Asn Asn Ile Met Pro Val Leu Glu Val Lys Ala Arg Arg Val  
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Gly Gly Ser Asn Tyr Gln Val Pro Val Glu Val Arg Pro Glu Arg Arg  
85 90 95

Thr Thr Leu Gly Leu Arg Trp Leu Val Asn Tyr Ala Arg Leu Arg Gly  
100 105 110

Glu Lys Thr Met Glu Asp Arg Leu Ala Asn Glu Ile Leu Asp Ala Ala  
115 120 125

Asn Asn Thr Gly Gly Ala Val Lys Lys Arg Glu Asp Thr His Lys Met  
130 135 140

Ala Glu Ala Asn Lys Ala Phe Ala His Tyr Arg Trp  
145 150 155

<210> 15  
<211> 615  
<212> DNA  
<213> Staphylococcus aureus

<400> 15  
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acaatgacag atccaatcgcc agatatgctt actcgtgtaa gaaacgc当地 catgggtcggt 180  
cacgagaagt tagaattacc tgcatcaaattt attaaaaaaag aaattgtc当地 aatcttaaag 240  
agtgaagggtt tcattaaaaa tggtgaatac gt当地 agatgatg ataaaacaagg tgtacttcgt 300  
ttattcttaa aatatggtca aaacgatgag cgtgttatca caggattaaa acgtatttca 360  
aaaccaggtt tacgtgttta tgcaaaaagct agc当地 gaaatgc ct当地 aaatggttta 420  
ggtattgc当地 tagtatcaac ttctgaaagggt gtaatcactg acaaaagaagc aagaaaaacgt 480  
aatgttggtg gagaattat cgc当地 atacgtt tggtataaaa aaataaggag gtgccataac 540  
atgagtc当地 ctg ttggtaagaa aattattgac atccctagtg acgtaacagt aacttttgat 600  
ggaaatcatg taact 615

<210> 16  
<211> 132  
<212> PRT  
<213> Staphylococcus aureus

<400> 16  
Met Thr Met Thr Asp Pro Ile Ala Asp Met Leu Thr Arg Val Arg Asn

	5		10		15
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20		25			30
ys Lys Glu Ile Ala Glu Ile Leu Lys Ser Glu Gly Phe Ile Lys Asn					
35		40			45
al Glu Tyr Val Glu Asp Asp Lys Gln Gly Val Leu Arg Leu Phe Leu					
50		55			60
ys Tyr Gly Gln Asn Asp Glu Arg Val Ile Thr Gly Leu Lys Arg Ile					
55		70			80
er Lys Pro Gly Leu Arg Val Tyr Ala Lys Ala Ser Glu Met Pro Lys					
85		90			95
al Leu Asn Gly Leu Gly Ile Ala Leu Val Ser Thr Ser Glu Gly Val					
100		105			110
le Thr Asp Lys Glu Ala Arg Lys Arg Asn Val Gly Gly Glu Ile Ile					
115		120			125
la Tyr Val Trp					
130					

<210> 17  
<211> 517  
<212> DNA  
<213> *Staphylococcus aureus*

<400> 17  
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gaacgtaaaa acgaaatcat taaagaatac cggtacacg aaactgatac tgttcacca 180  
gaagtacaaa tcgctgtact tactgcagaa atcaacgcag taaacgaaca cttagtaca 240  
cacaaaaaag accaccattc acgtcggttga ttatTTTTAA tggtaggtcg tcgttagacat 300  
ttatTTAAact acttacgttag taaagatatt caacgttacc gtgaattaat taaatcactt 360  
ggtatccgtc gttaatctta atataacgtc tttgaggTTG gggcatattt atgttccaac 420  
cttaatttat attaaaaaaag ctttttacaa atattaacat ttattatATG ttaagctaat 480  
atTgagtGaa taataaggTTT acaatgagat aaagatG 517

<210> 18  
<211> 89  
<212> PRT  
<213> *Staphylococcus aureus*

<400> 18  
Met Ala Ile Ser Gln Glu Arg Lys Asn Glu Ile Ile Lys Glu Tyr Arg  
1 5 10 15

Val His Glu Thr Asp Thr Gly Ser Pro Glu Val Gln Ile Ala Val Leu  
20 25 30

Thr Ala Glu Ile Asn Ala Val Asn Glu His Leu Arg Thr His Lys Lys  
35 40 45

Asp His His Ser Arg Arg Gly Leu Leu Lys Met Val Gly Arg Arg Arg  
50 55 60

His Leu Leu Asn Tyr Leu Arg Ser Lys Asp Ile Gln Arg Tyr Arg Glu  
 65                   70                   75                   80

Leu Ile Lys Ser Leu Gly Ile Arg Arg  
 85

<210> 19  
<211> 401  
<212> DNA  
<213> Staphylococcus aureus

<400> 19  
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gtttcagaca aaatggacaa gactattaca gtacttggtg aaacttacaa aacacacaaa 180  
ttatacggtt aacgagtaaa atactctaaa aaatacaaaa ctcatgatga aaacaattca 240  
gctaaattag gagacattgt taaaattcaa gaaactcggtc ctttacgc aacaaaacgt 300  
tttcgttttag tagagattgt tgaagagtca gtaatttattt aatacaagtt tagagataag 360  
ggagggtttaa ctaatgatcc aacaagaaac acgcttggaa g                   401

<210> 20  
<211> 87  
<212> PRT  
<213> Staphylococcus aureus

<400> 20  
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 1               5                   10                   15

Asp Lys Met Asp Lys Thr Ile Thr Val Leu Val Glu Thr Tyr Lys Thr  
 20               25                   30

His Lys Leu Tyr Gly Lys Arg Val Lys Tyr Ser Lys Lys Tyr Lys Thr  
 35               40                   45

His Asp Glu Asn Asn Ser Ala Lys Leu Gly Asp Ile Val Lys Ile Gln  
 50               55                   60

Glu Thr Arg Pro Leu Ser Ala Thr Lys Arg Phe Arg Leu Val Glu Ile  
 65               70                   75                   80

Val Glu Glu Ser Val Ile Ile  
 85

<210> 21  
<211> 1555  
<212> DNA  
<213> Staphylococcus aureus

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aatattttga accgcattgt tcaaaaagtga aagacggctc tgctgtcaact tatagatgga 240  
tccgcgtgc attagctgt tggtaaggtt acggcttacc aaggcaacga tacgttagccg 300  
acctgagagg gtgatcggcc acactggAAC tgagacacgg tccagactcc tacgggaggc 360  
agcagtaggg aatcttccgc aatggggcga agcctgacgg agcaacgcgg cgtgagtgat 420  
gaaggtcttc ggatcgtaaa actctgttat taggaaagaa catatgtta agtaactgtg 480  
cacatcttga cggtaacttac acgtgccaggc agccgcggta 540

atacgttagt	ggcaagcggt	atccggaaatt	attggggcgta	aagcgcgcgt	aggcggtttt	600
ttaagtctga	tgtgaaaagcc	cacggctcaa	cogtggaggg	tcattggaaa	ctggaaaaact	660
tgagtgccaga	agaggaaaagt	ggaattccat	gtgtagcggt	gaaatgcgca	gagatatgga	720
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agagatagag	cctccccctt	cgggggacaa	agtgacaggt	ggtgcatggt	tgctgtcagc	1080
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atcattaagt	tggcactct	aagttgactg	cogggtaccaa	accggaggaa	ggtggggatg	1200
acgtcaaatc	atcatgcccc	ttatgatttg	ggctacacac	gtgctacaat	ggacaataca	1260
aagggcagcg	aaaccgcgag	gtcaagcaaa	tcccataaaag	ttgttctcag	ttcggattgt	1320
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gtgaataacgt	tcccggttat	tgtacacacc	gcccggtcaca	ccacgagagt	ttgtAACacc	1440
cgaagccggt	ggagtaacct	tttaggagct	agccgtcgaa	ggtgggacaa	atgattgggg	1500
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<210> 22  
<211> 1294  
<212> DNA  
<213> *Staphylococcus aureus*

<400> 22  
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actggcgagg tacaacaagt tgaagacaag caagttgtt ttcatatcaa cggtggtaaa 180  
tttaatggga ttattcctat tagtcaacta tctacgcatt atattgatag cccaagtgaa 240  
gtttaaaaag agggcgacga agttgaagca tatgtcacta aagttgagtt tgatgaagaa 300  
aatgaaaactg gagcttacat cttatctaga agacaacttg aaactgagaa gtcttatagt 360  
tatttacaag aaaaattaga taataatgaa atcatcgaa cgaaaagtaac agaagttagtt 420  
aaaggtggtt tgggtgttga tgttaggacaa agagggttttgc ttccggcttc actaatttca 480  
acagacttca ttgaggattt ctctgtgtt gatggacaaa caattcgtat taaaagttgaa 540  
gaattggatc ctgaaaataa tagagtcatt ttaagccgta aagcagttga acaagaagaa 600  
aacgatgtca aaaaagatca attattacaa tctttaaatg aaggcgatgt tattgtatgg 660  
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gtgcattgtat ctgaaacttcc tcacgaacat gttcaaaacac cagaagaagt agtttcaattt 780  
ggtcaagatg taaaagttaa aattaaatct attgatagag atacagaacg tatttcattta 840  
tcaatcaaag atacgttacc aacacccccc gaaaatttta aaggtcaattt ccacgaaaat 900  
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aatgaaaagag tattcaatc tattaaagca acattacca acgaagatgt tgttgaaagt 1140  
gatccttcta cgactaaggc gtacttagaa aacgaagaag aagataatcc aacaatttgc 1200  
gatatgattt gtgataaact taaaaatctt aaactataat ttaatatttta atagtcaact 1260  
ccacatgtttt atgattqcat qtggacttatt tta 1294

<210> 23  
<211> 391  
<212> PRT  
<213> *Staphylococcus aureus*

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<400> 23
Met Thr Glu Glu Phe Asn Glu Ser Met Ile Asn Asp Ile Lys Glu Gly
      1           5           10          15

Asp Lys Val Thr Gly Glu Val Gln Gln Val Glu Asp Lys Gln Val Val
      20          25          30

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35	40	45
Leu Ser Thr His His Ile Asp Ser Pro Ser Glu Val Val Lys Glu Gly		
50	55	60
Asp Glu Val Glu Ala Tyr Val Thr Lys Val Glu Phe Asp Glu Glu Asn		
65	70	75
80		
Glu Thr Gly Ala Tyr Ile Leu Ser Arg Arg Gln Leu Glu Thr Glu Lys		
85	90	95
Ser Tyr Ser Tyr Leu Gln Glu Lys Leu Asp Asn Asn Glu Ile Ile Glu		
100	105	110
Ala Lys Val Thr Glu Val Val Lys Gly Gly Leu Val Val Asp Val Gly		
115	120	125
Gln Arg Gly Phe Val Pro Ala Ser Leu Ile Ser Thr Asp Phe Ile Glu		
130	135	140
Asp Phe Ser Val Phe Asp Gly Gln Thr Ile Arg Ile Lys Val Glu Glu		
145	150	155
160		
Leu Asp Pro Glu Asn Asn Arg Val Ile Leu Ser Arg Lys Ala Val Glu		
165	170	175
Gln Glu Glu Asn Asp Ala Lys Lys Asp Gln Leu Leu Gln Ser Leu Asn		
180	185	190
Glu Gly Asp Val Ile Asp Gly Lys Val Ala Arg Leu Thr Gln Phe Gly		
195	200	205
Ala Phe Ile Asp Ile Gly Gly Val Asp Gly Leu Val His Val Ser Glu		
210	215	220
Leu Ser His Glu His Val Gln Thr Pro Glu Glu Val Val Ser Ile Gly		
225	230	235
240		
Gln Asp Val Lys Val Lys Ile Lys Ser Ile Asp Arg Asp Thr Glu Arg		
245	250	255
Ile Ser Leu Ser Ile Lys Asp Thr Leu Pro Thr Pro Phe Glu Asn Ile		
260	265	270
Lys Gly Gln Phe His Glu Asn Asp Val Ile Glu Gly Val Val Val Arg		
275	280	285
Leu Ala Asn Phe Gly Ala Phe Val Glu Ile Ala Pro Gly Val Gln Gly		
290	295	300
Leu Val His Ile Ser Glu Ile Ala His Lys His Ile Gly Thr Pro Gly		
305	310	315
320		
Glu Val Leu Glu Pro Gly Gln Gln Val Asn Val Lys Ile Leu Glu Ile		
325	330	335
Asp Glu Glu Asn Glu Arg Val Ser Leu Ser Ile Lys Ala Thr Leu Pro		
340	345	350
Asn Glu Asp Val Val Glu Ser Asp Pro Ser Thr Thr Lys Ala Tyr Leu		
355	360	365

Glu Asn Glu Glu Asp Asn Pro Thr Ile Gly Asp Met Ile Gly Asp  
 370                   375                   380

Lys Leu Lys Asn Leu Lys Leu  
 385                   390

<210> 24  
<211> 924  
<212> DNA  
<213> Staphylococcus aureus

<400> 24  
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cgttggaaacc caaaaaatgaa aaaatatac ttcaactgaga gaaatggtat ttatataatc 180  
gacttacaaa aaacagtgaa aaaagtagac gaggcataca acttcttggaa acaagttca 240  
gaagatggtg gacaagtctt attcgttagga nctaaaaaac aagcacaaga atcagttaaa 300  
tctgaagcag aacgtgctgg tcaattctac attaaccaaa gatggttagg tggattatta 360  
actaactata aaacgatctc aaaacgaatc aaacgtatcc ctgaaatgaa aaaaatggaa 420  
gaagatggtt tattcgaagt attacctaaa aaagaagtag tagaacttaa aaaaagaatac 480  
gaccgtttaa tcaaattctt aggcggatt cgtgatatga aatcaatgcc tcaagcatta 540  
ttcgttagtg acccacgtaa agagcgtaat gcaattgctg aagctcgtaa attaaatatt 600  
cctatcgtag gtatcggtga cactaactgt gatcctgacg aaattgacta cgttatccca 660  
gcaaacgacg atgctatccg tgccgtttaaa ttattaactg ctaaaatggc agatgcaatc 720  
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gatgaaaaaaag aaaaatcaga agaaacagaa gcaactgaag aataatcaac tggtaatct 840  
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<210> 25  
<211> 255  
<212> PRT  
<213> Staphylococcus aureus

<400> 25  
Met Ala Val Ile Ser Met Lys Gln Leu Leu Glu Ala Gly Val Xaa Phe  
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Gly His Gln Thr Arg Arg Trp Asn Pro Lys Met Lys Lys Tyr Ile Phe  
 20                   25                   30

Thr Glu Arg Asn Gly Ile Tyr Ile Ile Asp Leu Gln Lys Thr Val Lys  
 35                   40                   45

Lys Val Asp Glu Ala Tyr Asn Phe Leu Lys Gln Val Ser Glu Asp Gly  
 50                   55                   60

Gly Gln Val Leu Phe Val Gly Thr Lys Lys Gln Ala Gln Glu Ser Val  
 65                   70                   75                   80

Lys Ser Glu Ala Glu Arg Ala Gly Gln Phe Tyr Ile Asn Gln Arg Trp  
 85                   90                   95

Leu Gly Gly Leu Leu Thr Asn Tyr Lys Thr Ile Ser Lys Arg Ile Lys  
 100                105                110

Arg Ile Ser Glu Ile Glu Lys Met Glu Glu Asp Gly Leu Phe Glu Val  
 115                120                125

Leu Pro Lys Lys Glu Val Val Glu Leu Lys Lys Glu Tyr Asp Arg Leu

130	135	140
-----	-----	-----

Ile Lys Phe Leu Gly Gly Ile Arg Asp Met Lys Ser Met Pro Gln Ala	145	150	155	160
---	-----	-----	-----	-----

Leu Phe Val Val Asp Pro Arg Lys Glu Arg Asn Ala Ile Ala Glu Ala	165	170	175
---	-----	-----	-----

Arg Lys Leu Asn Ile Pro Ile Val Gly Ile Val Asp Thr Asn Cys Asp	180	185	190
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Pro Asp Glu Ile Asp Tyr Val Ile Pro Ala Asn Asp Asp Ala Ile Arg	195	200	205
---	-----	-----	-----

Ala Val Lys Leu Leu Thr Ala Lys Met Ala Asp Ala Ile Leu Glu Gly	210	215	220
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Gln Gln Gly Val Ser Asn Glu Glu Val Ala Ala Glu Gln Asn Ile Asp	225	230	235	240
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Leu Asp Glu Lys Glu Lys Ser Glu Glu Thr Glu Ala Thr Glu Glu	245	250	255
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&lt;210&gt; 26

&lt;211&gt; 800

&lt;212&gt; DNA

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 26

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ttttacacga agataaaaaa atccgtaaat ttattgataa tgaattaaaa gaagcatcag 240	tttctcacgt agagattgaa cgtgctgcaa accgtatcaa cattgcaatt catactggta 300	tttctcacgt agagattgaa cgtgctgcaa accgtatcaa cattgcaatt catactggta 360
aacctggat ggtatggg aaaggcggtt cagaaaatcgaa aaaattacgc aacaaattaa 420	atgcgttaac tgataaaaaaa gtacacatca acgttaattga aataaaaaaaaa gttgatctt 480	acgctcggtt agtagctgaa aacatcgac gtcaatttga aaaccgtgct tcattccgtc 540
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cttacggtaa attaggcggtt aaagtatggaa ttatcggtt agaagttttt cctactaaga 780	acactagttgg aggaggaaaa taataatgtt actacaaaaa cgtgtaaaat atcgctgtca 800	acatcgctt aaaaacaactg

&lt;210&gt; 27

&lt;211&gt; 221

&lt;212&gt; PRT

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 27

Met Gly Asn Thr Val Gly Gln Lys Ile Asn Pro Ile Gly Leu Arg Val	1	5	10	15
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Gly Ile Ile Arg Asp Trp Glu Ala Lys Trp Tyr Ala Glu Lys Asp Phe	20	25	30
---	----	----	----

Ala Ser Leu Leu His Glu Asp Leu Lys Ile Arg Lys Phe Ile Asp Asn	35	40	45
---	----	----	----

Glu Leu Lys Glu Ala Ser Val Ser His Val Glu Ile Glu Arg Ala Ala		
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50	55	60
Asn Arg Ile Asn Ile Ala Ile His Thr Gly Lys Pro Gly Met Val Ile		
65	70	75
Gly Lys Gly Ser Glu Ile Glu Lys Leu Arg Asn Lys Leu Asn Ala		
85	90	95
Leu Thr Asp Lys Lys Val His Ile Asn Val Ile Glu Ile Lys Lys Val		
100	105	110
Asp Leu Asp Ala Arg Leu Val Ala Glu Asn Ile Ala Arg Gln Leu Glu		
115	120	125
Asn Arg Ala Ser Phe Arg Arg Val Gln Lys Gln Ala Ile Thr Arg Ala		
130	135	140
Met Lys Leu Gly Ala Lys Gly Ile Lys Thr Gln Val Ser Gly Arg Leu		
145	150	155
160		
Gly Gly Ala Asp Ile Ala Arg Ala Glu Gln Tyr Ser Glu Gly Thr Val		
165	170	175
Pro Leu His Thr Leu Arg Ala Asp Ile Asp Tyr Ala His Ala Glu Ala		
180	185	190
Asp Thr Thr Tyr Gly Lys Leu Gly Val Lys Val Trp Ile Tyr Arg Gly		
195	200	205
Glu Val Leu Pro Thr Lys Asn Thr Ser Gly Gly Lys		
210	215	220

<210> 28  
<211> 639  
<212> DNA  
<213> *Staphylococcus aureus*

<400> 28  
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gttgttacaa tcaaccgtgt agcaaaagtt gtaaaaaggtg gtcgtcgctt ccgtttcact 180  
gcattagttg tagttggaga caaaaatggt cgttaggtt tcggtaactgg taaagctcaa 240  
gaggtaccag aagcaatcaa aaaagctgtt gaagcagcta aaaaagattt agtagttgtt 300  
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tttatgaaac cggctgcacc tggtacagga gttatcgctg gtggccctgt tcgtgccgtt 420  
cttgaattag caggtatcac tgatatctt agtaaatcat taggatcaaa cacaccaatc 480  
aacatggttc gtgctacaat cgatggttt caaaacctt aaaaatgcgtt agatgttgcg 540  
aaattacgtt gcaaaacagt agaagaatta tacaattaag gagggaaaac tagttatggc 600  
taaattacaa attaccctca ctcgtatgtt tattggtcg 639

<210> 29  
<211> 166  
<212> PRT  
<213> *Staphylococcus aureus*

<400> 29  
Met Ala Arg Arg Glu Glu Glu Thr Lys Glu Phe Glu Glu Arg Val Val  
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Thr Ile Asn Arg Val Ala Lys Val Val Lys Gly Gly Arg Arg Phe Arg

20

25

30

Phe Thr Ala Leu Val Val Val Gly Asp Lys Asn Gly Arg Val Gly Phe  
 35 40 45

Gly Thr Gly Lys Ala Gln Glu Val Pro Glu Ala Ile Lys Lys Ala Val  
 50 55 60

Glu Ala Ala Lys Lys Asp Leu Val Val Val Pro Arg Val Glu Gly Thr  
 65 70 75 80

Thr Pro His Thr Ile Thr Gly Arg Tyr Gly Ser Gly Ser Val Phe Met  
 85 90 95

Lys Pro Ala Ala Pro Gly Thr Gly Val Ile Ala Gly Gly Pro Val Arg  
 100 105 110

Ala Val Leu Glu Leu Ala Gly Ile Thr Asp Ile Leu Ser Lys Ser Leu  
 115 120 125

Gly Ser Asn Thr Pro Ile Asn Met Val Arg Ala Thr Ile Asp Gly Leu  
 130 135 140

Gln Asn Leu Lys Asn Ala Glu Asp Val Ala Lys Leu Arg Gly Lys Thr  
 145 150 155 160

Val Glu Glu Leu Tyr Asn  
 165

<210> 30

<211> 499

<212> DNA

<213> Staphylococcus aureus

<400> 30

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 atgtacatcg tacccccaaa cattgagaa gatgctaaaa aagcgttagt tgaacgtttc 180  
 aacggtatct tagctactga aggtgcagaa gttttagaag caaaagactg gggtaaacgt 240  
 cgcctagctt atgaaatcaa tgattcaaa gatggcttct acaacatcg acgtgttaaa 300  
 tctgataaca acaaagctac tgacgaattc caacgtctag ctaaaatcag tgacgatatac 360  
 attcgttaca tggttattcg tgaagacgaa gacaagtaat aatttagaggg ggcgtttaaa 420  
 tgctaaatag agttgttata gtaggtcggt taacgaaaga tccggaatac agaaccactc 480  
 cctcagggtgt gagtgtac 499

<210> 31

<211> 98

<212> PRT

<213> Staphylococcus aureus

<400> 31

Met Arg Thr Tyr Glu Val Met Tyr Ile Val Arg Pro Asn Ile Glu Glu  
 1 5 10 15

Asp Ala Lys Lys Ala Leu Val Glu Arg Phe Asn Gly Ile Leu Ala Thr  
 20 25 30

Glu Gly Ala Glu Val Leu Glu Ala Lys Asp Trp Gly Lys Arg Arg Leu  
 35 40 45

Ala Tyr Glu Ile Asn Asp Phe Lys Asp Gly Phe Tyr Asn Ile Val Arg  
 50 55 60

Val Lys Ser Asp Asn Asn Lys Ala Thr Asp Glu Phe Gln Arg Leu Ala  
 65 70 75 80

Lys Ile Ser Asp Asp Ile Ile Arg Tyr Met Val Ile Arg Glu Asp Glu  
 85 90 95

Asp Lys

<210> 32

<211> 462

<212> DNA

<213> Staphylococcus aureus

<400> 32

gtgcacaaca accagaaaaac tacgaattac gtggtaatt agaaggagga aatgactttg 60  
 gcacaagttg aatatagagg cacaggccgt cgtaaaaact cagtagcacg tgcgttta 120  
 gtaccagggtg aaggtAACAT cacagttaat aaccgtgacg tacgcata cttaccattc 180  
 gaatcattaa tttagactt aaaccaacca ttgtatgtaa ctgaaactaa aggttaactat 240  
 gatgttttag ttaacgttca tggtggttgtt ttcactggac aagctcaagc tatccgtcac 300  
 ggaatcgctc gtgcattatt agaagcagat cctgaataca gaggttcttt aaaacgcgtc 360  
 ggattactta ctcgtgaccc acgtatgaaa gaacgtaaaa aaccaggctct taaaggcagct 420  
 cgtcggttac acgttcaaaaatcttca ttgtcgacg at 462

<210> 33

<211> 132

<212> PRT

<213> Staphylococcus aureus

<400> 33

Met Thr Leu Ala Gln Val Glu Tyr Arg Gly Thr Gly Arg Arg Lys Asn  
 1 5 10 15

Ser Val Ala Arg Val Arg Leu Val Pro Gly Glu Gly Asn Ile Thr Val  
 20 25 30

Asn Asn Arg Asp Val Arg Glu Tyr Leu Pro Phe Glu Ser Leu Ile Leu  
 35 40 45

Asp Leu Asn Gln Pro Phe Asp Val Thr Glu Thr Lys Gly Asn Tyr Asp  
 50 55 60

Val Leu Val Asn Val His Gly Gly Gly Phe Thr Gly Gln Ala Gln Ala  
 65 70 75 80

Ile Arg His Gly Ile Ala Arg Ala Leu Leu Glu Ala Asp Pro Glu Tyr  
 85 90 95

Arg Gly Ser Leu Lys Arg Ala Gly Leu Leu Thr Arg Asp Pro Arg Met  
 100 105 110

Lys Glu Arg Lys Lys Pro Gly Leu Lys Ala Ala Arg Arg Ser Pro Gln  
 115 120 125

Phe Ser Lys Arg  
 130

<210> 34  
<211> 441  
<212> DNA  
<213> *Staphylococcus aureus*

<400> 34  
aggtaactga cacacccggc cgcttgcca tggcgctgtg taagatagtt ttgcgtggaga 60  
agtctatcac taaatgtaga cgaataagga gggaaaatta tggcaaaaca aaaaatcaga 120  
atcagattaa aagcttatga tcaccgcgtt attgtatcaat cagcagagaa gattgttagaa 180  
acagcgttac acgttttttgc agatgtttct ggaccaattc cggttaccaac tgagaaatca 240  
cgtacacaca aacgtttaat cgtatattgtt aacccaacac caaaaacagt tgacgcttta 300  
atgggcttaa acttaccatc tggtgttagac atcggaaatca aattataata gacaattttt 360  
ggaggtggac tttcgatgac caaaggaatc ttaggaagaa aaattggat gacacaagta 420  
ttcggagaaaa acggtaattt a 441

<210> 35  
<211> 102  
<212> PRT  
<213> *Staphylococcus aureus*

<400> 35  
Met Ala Lys Gln Lys Ile Arg Ile Arg Leu Lys Ala Tyr Asp His Arg  
1 5 10 15  
Val Ile Asp Gln Ser Ala Glu Lys Ile Val Glu Thr Ala Lys Arg Ser  
20 25 30  
Gly Ala Asp Val Ser Gly Pro Ile Pro Leu Pro Thr Glu Lys Ser Val  
35 40 45  
Tyr Thr Ile Ile Arg Ala Val His Lys Tyr Lys Asp Ser Arg Glu Gln  
50 55 60  
Phe Glu Gln Arg Thr His Lys Arg Leu Ile Asp Ile Val Asn Pro Thr  
65 70 75 80  
Pro Lys Thr Val Asp Ala Leu Met Gly Leu Asn Leu Pro Ser Gly Val  
85 90 95  
Asp Ile Glu Ile Lys Leu  
100

<210> 36  
<211> 594  
<212> DNA  
<213> *Staphylococcus aureus*

<400> 36  
agttcgttgtt caaaaaacga aaaacmacgc gcgtactcgt aaaggaccag ttaaaaacggt 60  
agctaacaag aaaaaatmat aggttaaagga ggcaaatttt aaatggcagc taaaacaagta 120  
tctcgtaaac gttagtgaa aaagaatatt gaaaatggtg tagcacacat ccgttcaaca 180  
ttcaacaaca ctattgtAAC tatcaactgtat gagtcggta atgcattatc atggcatca 240  
gctgggtcat taggattcaa aggatctaaa aaatcaacac catttgcagc acaaatggct 300  
tctgaaactg catctaaatc agctatggag catgtttaa aaacagtta agtaacagtt 360  
aaaggacctg gtccaggtcg tgaatcagct attcgtgcatt tacaatctgc aggttttagaa 420  
gttaactgcga tcagagacgt tactccagta cctcataacg gttgtcgcc accaaaacgt 480  
cgtcgtgtat aatttatgtat ggtattgtt caggtcactg agcaaaacatt ttaaattaag 540  
tcgacgtata taaggaggat atttaaatga tagaaatcga aaaacctaga attg 594

<210> 37  
<211> 129  
<212> PRT  
<213> Staphylococcus aureus

<400> 37  
Met Ala Arg Lys Gln Val Ser Arg Lys Arg Arg Val Lys Lys Asn Ile  
1               5               10               15  
Glu Asn Gly Val Ala His Ile Arg Ser Thr Phe Asn Asn Thr Ile Val  
20               25               30  
Thr Ile Thr Asp Glu Phe Gly Asn Ala Leu Ser Trp Ser Ser Ala Gly  
35               40               45  
Ala Leu Gly Phe Lys Gly Ser Lys Lys Ser Thr Pro Phe Ala Ala Gln  
50               55               60  
Met Ala Ser Glu Thr Ala Ser Lys Ser Ala Met Glu His Gly Leu Lys  
65               70               75               80  
Thr Val Glu Val Thr Val Lys Gly Pro Gly Pro Gly Arg Glu Ser Ala  
85               90               95  
Ile Arg Ala Leu Gln Ser Ala Gly Leu Glu Val Thr Ala Ile Arg Asp  
100              105              110  
Val Thr Pro Val Pro His Asn Gly Cys Arg Pro Pro Lys Arg Arg Arg  
115              120              125  
Val

<210> 38  
<211> 620  
<212> DNA  
<213> Staphylococcus aureus

<400> 38  
ttaaatgaga attagtaagt gttttactta ctaaatttttta tttaacctaa aaatgaacca 60  
cctggatgtg tgggattaaa aagtgaagag aggaggacat atcacatgcc aactattaac 120  
caatttagtac gttaaaccaag acaaagcaaa atcaaaaaat cagattctcc agctttaaat 180  
aaagggttca acagaaaaaa gaaaaaattt actgacttta actcaccaca aaaacgtgg 240  
gtatgtactc gtgttaggtac aatgacacct aaaaaaccta actcagcggtt acgtaaaatat 300  
gcacgtgtgc gtttatcaaa caacatcgaa attaacgcacat acatccctgg tatcggacat 360  
aacttacaag aacacagtgt tgtacttgta cgtggtgac 420  
gtgcgttacc atattgtacg tggagcactt gataacttcag gtgttgacgg acgtagacaa 480  
gtgcgttcat tatacggAAC taagaaacct aaaaactaag aatttagttt ttaattaaat 540  
cttaaactta aaatatttaa tataaggAAC ggaggattt cattatgcct cgtaaaggat 600  
cagtacctaa aagagacgtt 620

<210> 39  
<211> 137  
<212> PRT  
<213> Staphylococcus aureus

<400> 39  
Met Pro Thr Ile Asn Gln Leu Val Arg Lys Pro Arg Gln Ser Lys Ile  
1               5               10               15

Lys Lys Ser Asp Ser Pro Ala Leu Asn Lys Gly Phe Asn Ser Lys Lys  
 20 25 30

Lys Lys Phe Thr Asp Leu Asn Ser Pro Gln Lys Arg Gly Val Cys Thr  
 35 40 45

Arg Val Gly Thr Met Thr Pro Lys Lys Pro Asn Ser Ala Leu Arg Lys  
 50 55 60

Tyr Ala Arg Val Arg Leu Ser Asn Asn Ile Glu Ile Asn Ala Tyr Ile  
 65 70 75 80

Pro Gly Ile Gly His Asn Leu Gln Glu His Ser Val Val Leu Val Arg  
 85 90 95

Gly Gly Arg Val Lys Asp Leu Pro Gly Val Arg Tyr His Ile Val Arg  
 100 105 110

Gly Ala Leu Asp Thr Ser Gly Val Asp Gly Arg Arg Gln Gly Arg Ser  
 115 120 125

Leu Tyr Gly Thr Lys Lys Pro Lys Asn  
 130 135

&lt;210&gt; 40

&lt;211&gt; 633

&lt;212&gt; DNA

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 40

gtataaaaat gaaagtaaga ccatcagtaa aacctatttg cgaaaaatgt aaagtcatta 60  
 aacgtaaagg taaagtaatg gtaatttgcg aaaatccaa acacaaacaa agacaagggtt 120  
 aataaaaagag aggtgtaaat taatatggca cgtattgcag gagtagatat tccacgtgaa 180  
 aaacgcgtag ttatctcatt aacttatata tacggtatcg gtacgtcaac tgctcaaaaa 240  
 attcttgaag aagctaaccgt atcagctgat actcgtgtga aagatttaac tgatgacgaa 300  
 ttaggtcgca tccgtgaagt ttttagacggt tataaagtgcg aagggtgactt acgtcgtgaa 360  
 actaacttaa atatcaaaccg tttaatggaa atttcatcat accgtggat ccgtcaccgt 420  
 cgtggtttac cagttcgtgg tcaaaaaacgc aaaaacaacg cgcgtactcg taaaggacca 480  
 gttaaaacgg tagctaacaa gaaaaaaataa tagttaaagg aggcaaattt taaatggcac 540  
 gtaaacaagt atctcgtaaa cgttagatgtaaaaggatat tgaaaatgggt gtagcacaca 600  
 tccgttcaac attcaacaac actattgtaa cta 633

&lt;210&gt; 41

&lt;211&gt; 121

&lt;212&gt; PRT

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 41

Met Ala Arg Ile Ala Gly Val Asp Ile Pro Arg Glu Lys Arg Val Val  
 1 5 10 15

Ile Ser Leu Thr Tyr Ile Tyr Gly Ile Gly Thr Ser Thr Ala Gln Lys  
 20 25 30

Ile Leu Glu Glu Ala Asn Val Ser Ala Asp Thr Arg Val Lys Asp Leu  
 35 40 45

Thr Asp Asp Glu Leu Gly Arg Ile Arg Glu Val Val Asp Gly Tyr Lys  
 50 55 60

Val Glu Gly Asp Leu Arg Arg Glu Thr Asn Leu Asn Ile Lys Arg Leu  
65 70 75 80

Met Glu Ile Ser Ser Tyr Arg Gly Ile Arg His Arg Arg Gly Leu Pro  
85 90 95

Val Arg Gly Gln Lys Thr Lys Asn Asn Ala Arg Thr Arg Lys Gly Pro  
100 105 110

Val Lys Thr Val Ala Asn Lys Lys Lys  
115 120

<210> 42  
<211> 311  
<212> DNA  
<213> *Staphylococcus aureus*

<400> 42  
ctcgtaatt gtagctaac ttccgtatgc cattccgtaa ataattattt aaaggaggct 60  
aattaagtgg ctaaaacttc aatggttgct aagcaacaaa aaaaacaaaa atatgcagtt 120  
cgtgaataaca ctctgtgtga acgttggc cgtccacatt ctgtatatcg taaatttaaa 180  
ttatgccgta ttgtttccg tgaattagct tacaaaggcc aaatccctgg cggtcgtaaa 240  
gctagcttgt aataaaaaaag agtctgaaag gaggcaacaa tcaatgacaa tgacagatcc 300  
aatcgacat a 311

<210> 43  
<211> 61  
<212> PRT  
<213> *Staphylococcus aureus*

<400> 43  
Met Ala Lys Thr Ser Met Val Ala Lys Gln Gln Lys Lys Gln Lys Tyr  
1 5 10 15

Ala Val Arg Glu Tyr Thr Arg Cys Glu Arg Cys Gly Arg Pro His Ser  
20 25 30

Val Tyr Arg Lys Phe Lys Leu Cys Arg Ile Cys Phe Arg Glu Leu Ala  
35 40 45

Tyr Lys Gly Gln Ile Pro Gly Val Arg Lys Ala Ser Trp  
50 55 60

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<210> 44
<211> 710
<212> DNA
<213> Staphylococcus aureus
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<400> 44
aacattcata caccgtttaa tattatttct ttagaaaaat aaaaattaaa acatgactta 60
aaggagatt tataaatggc agttaaaatt cgtttaacac gtttagtttc aaaaagaaaat 120
ccattctatc gtatcgtagt agcagatgct cgttctccac gtgacgacg tatcatcgaa 180
caaatcggtt ctatataaccc aacgagcgct aatgctccag aaattaaagt tgacgaagcg 240
ttagcttaa aatggttaaa tgatggtgcg aaacccaactg atacaggttca caatatctta 300
tcaaaaagaag gtatttatgaa aaaatttgac gaacaaaaga aagctaagta atttagcgta 360
aaattgttct aacaataaga ataactcggt tacactgaca gtttattactc aatgataacgt 420
tgggaatatc acatgttagt aatatagaac gtttgggtac cataatggtg cccttttct 480
ttgaattatt ttcaattaaa atagaagtgg tcaaagcata gagttggagg taatagaatg 540
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agagttgaag ttggtaaaaa ttgtttacac acacggggtt taaaagggtgg aaattaaagg 600  
 taaatccatt tcagacctt tacagaccgg ttcggtttc aaccccggtc caaagatgcc 660  
 tgaccagttg ggccttaaac caaattaaac cgaccccctt ggaaatatta 710

<210> 45  
 <211> 92  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 45  
 Met Ala Val Lys Ile Arg Leu Thr Arg Leu Gly Ser Lys Arg Asn Pro  
 1 5 10 15

Phe Tyr Arg Ile Ile Val Val Ala Asp Ala Arg Ser Pro Arg Asp Gly  
 20 25 30

Arg Ile Ile Glu Gln Ile Gly Thr Tyr Asn Pro Thr Ser Ala Asn Ala  
 35 40 45

Pro Glu Ile Lys Val Asp Glu Ala Leu Ala Leu Lys Trp Leu Asn Asp  
 50 55 60

Gly Ala Lys Pro Thr Asp Thr Val His Asn Ile Leu Ser Lys Glu Gly  
 65 70 75 80

Ile Met Lys Lys Phe Asp Glu Gln Lys Lys Ala Lys  
 85 90

<210> 46  
 <211> 437  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 46  
 aatgcaaacg gaccgattga tataagtcat gatgacttac cattctaata aaaattaacg 60  
 aaattaaacg gaaaaaaatta tcaaaggagg cacacaatca tggcagggtgg accaagaaga 120  
 ggcggacgtc gtcgtaaaaa agttagtcat ttcacagcaa atggtattac acatatcgac 180  
 tacaagaca ctgaattatt aaaacgtttt atctcagaac gcggtaaaat tttaccacgt 240  
 cgtgtaactg gtacttcagc taaaatatcaa cgtatgttg ctacagctat caaacgttct 300  
 cgtcatatgg cattattacc atatgttaaa gaagaacaat aatatataat ttattgtcaa 360  
 accccgttagg cataggctta cggggcttt tgggtttgg ggtatagaaa aaggcggaaaa 420  
 aggatgatgtt gaatgtt 437

<210> 47  
 <211> 80  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 47  
 Met Ala Gly Gly Pro Arg Arg Gly Arg Arg Arg Lys Lys Val Cys  
 1 5 10 15

Tyr Phe Thr Ala Asn Gly Ile Thr His Ile Asp Tyr Lys Asp Thr Glu  
 20 25 30

Leu Leu Lys Arg Phe Ile Ser Glu Arg Gly Lys Ile Leu Pro Arg Arg  
 35 40 45

Val Thr Gly Thr Ser Ala Lys Tyr Gln Arg Met Leu Thr Thr Ala Ile

50	55	60
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Lys	Arg	Ser	Arg	His	Met	Ala	Leu	Leu	Pro	Tyr	Val	Lys	Glu	Glu	Gln
65												75			80

<210> 48  
<211> 478  
<212> DNA  
<213> Staphylococcus aureus

<400> 48  
aaacttacg ttcgtggacg taagaaaaaa taatataatc aacttatttg ggtgtgcggc 60  
ttaaagctgc acgcacataa taagaaggga ggcccccggaa tggctcgtag tattaaaaaa 120  
ggacctttcg tcgatgagca ttatgaaa aaagttgaag ctcaagaagg aagcgaaaag 180  
aaacaagtaa tcaaaacatg gtcacgtcgt tctacaattt tccctaattt catcggacat 240  
acttttgcag tatacgacgg acgtaaacac gtacctgtat atgtactga agatatggta 300  
ggtcataaat tagtgagtt tgctcctact cgtacattca aaggacacgt tgcagacgac 360  
aagaaaacaa gaagataata tctattaatg agaggaggac atcctaattgg aagcaaaagc 420  
ggttgctaga acaataagaa tcgcacctcg taaagtaaga ctgttcttg acttaatc 478

<210> 49  
<211> 92  
<212> PRT  
<213> Staphylococcus aureus

<400> 49  
Met Ala Arg Ser Ile Lys Lys Gly Pro Phe Val Asp Glu His Leu Met  
1 5 10 15

Lys Lys Val Glu Ala Gln Glu Gly Ser Glu Lys Lys Gln Val Ile Lys  
20 25 30

Thr Trp Ser Arg Arg Ser Thr Ile Phe Pro Asn Phe Ile Gly His Thr  
35 40 45

Phe Ala Val Tyr Asp Gly Arg Lys His Val Pro Val Tyr Val Thr Glu  
50 55 60

Asp Met Val Gly His Lys Leu Gly Glu Phe Ala Pro Thr Arg Thr Phe  
65 70 75 80

Lys Gly His Val Ala Asp Asp Lys Lys Thr Arg Arg  
85 90

<210> 50  
<211> 520  
<212> DNA  
<213> Staphylococcus aureus

<400> 50  
tgcaaaaattt taagctaacc ccatcaaata aatgattgca caacggtagt acttttgttta 60  
aaatatattct tggtaatc aaataaaatt ttgataagat gaactcactt ttaggaggtg 120  
gcagaaaatgg caaatatcaa atctgcaatt aaacgtgtaa aaacaactga aaaagctgaa 180  
gcacgcaaca tttcacaaaa gagtgcaatg cgtacagcag taaaaacgc taaaacagct 240  
gtttcaaaaata acgctgataa taaaaatgaa tttagtaagct tagcagttaa gtttagtagac 300  
aaagctgctc aaagtaattt aatacattca aacaaagctg accgtattaa atcacaatta 360

atgactgcaa ataaataatc tttttaaata aaagttcaag cgcatgcgg aacttttatt 420  
 ttttataaag atagaatgaa taattccagt attaactgtt tatccatata tgatgattta 480  
 agtttataat cagttccgc acaagcatct ataatatattca 520

<210> 51  
 <211> 499  
 <212> DNA  
 <213> *Staphylococcus aureus*

<400> 51  
 tgtttcaaat aaaaaacaat ttactaattt accataaattt acagatataat tataacttata 60  
 aatgcatagt tttactgtgc aatttgcattt aaagttccgt tgatatttgg agggagggaa 120  
 atacagatgt cttaaacagt agtacgtaaa aatgaatcac ttgaagatgc gttacgtaga 180  
 tttaaacgtt cagtttctaa aagtggaaaca atccaagaag tacgtaaacg tgaattttac 240  
 gaaaaaccaa gcgtaaaacg taaaaagaaa tcagaagctg cacgtaaacg taaattcaaa 300  
 taattaatac ctctgttgc tccctcaaca cgaatattaa ttatataaaa caaacatcac 360  
 aagtttagtgt ctgacactaa tatgtgatgt tttttgttg tcaattttta attaaaaaaaa 420  
 gtttatatagt ttataaataa tcaaatttgc attctatagg ttcttataac tataaagtat 480  
 attcaatttc atgtataat 499

<210> 52  
 <211> 58  
 <212> PRT  
 <213> *Staphylococcus aureus*

<400> 52  
 Met Ser Lys Thr Val Val Arg Lys Asn Glu Ser Leu Glu Asp Ala Leu  
 1 5 10 15

Arg Arg Phe Lys Arg Ser Val Ser Lys Ser Gly Thr Ile Gln Glu Val  
 20 25 30

Arg Lys Arg Glu Phe Tyr Glu Lys Pro Ser Val Lys Arg Lys Lys Lys  
 35 40 45

Ser Glu Ala Ala Arg Lys Arg Lys Phe Lys  
 50 55

<210> 53  
 <211> 31  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:PCR Primer

<400> 53  
 tatattatcg ataatggctc gattcagagg t 31

<210> 54  
 <211> 36  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:PCR Primer

<400> 54

tataggatcc ttaacggatt aattgttcgt taattt 36

<210> 55  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:PCR Primer

<400> 55  
tatattatcg ataatggcag gtggaccaag aag 33

<210> 56  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:PCR Primer

<400> 56  
tataggatcc ttattgttct tctttAACAT 30

<210> 57  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:PCR Primer

<400> 57  
tatattatcg ataatgaaga aacatatgaa gttat 35

<210> 58  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:PCR Primer

<400> 58  
tataggatcc ttacttgtct tcgtttcac 30

<210> 59  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:PCR Primer

<400> 59  
caccacgaga gtttgtaac

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<210> 60
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR Primer

<400> 60
caccccaatc atttgtccccac

<210> 61
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR Primer

<400> 61
cacgtggata acctaccta

<210> 62
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR Primer

<400> 62
gtggccgatc accctctcagg
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